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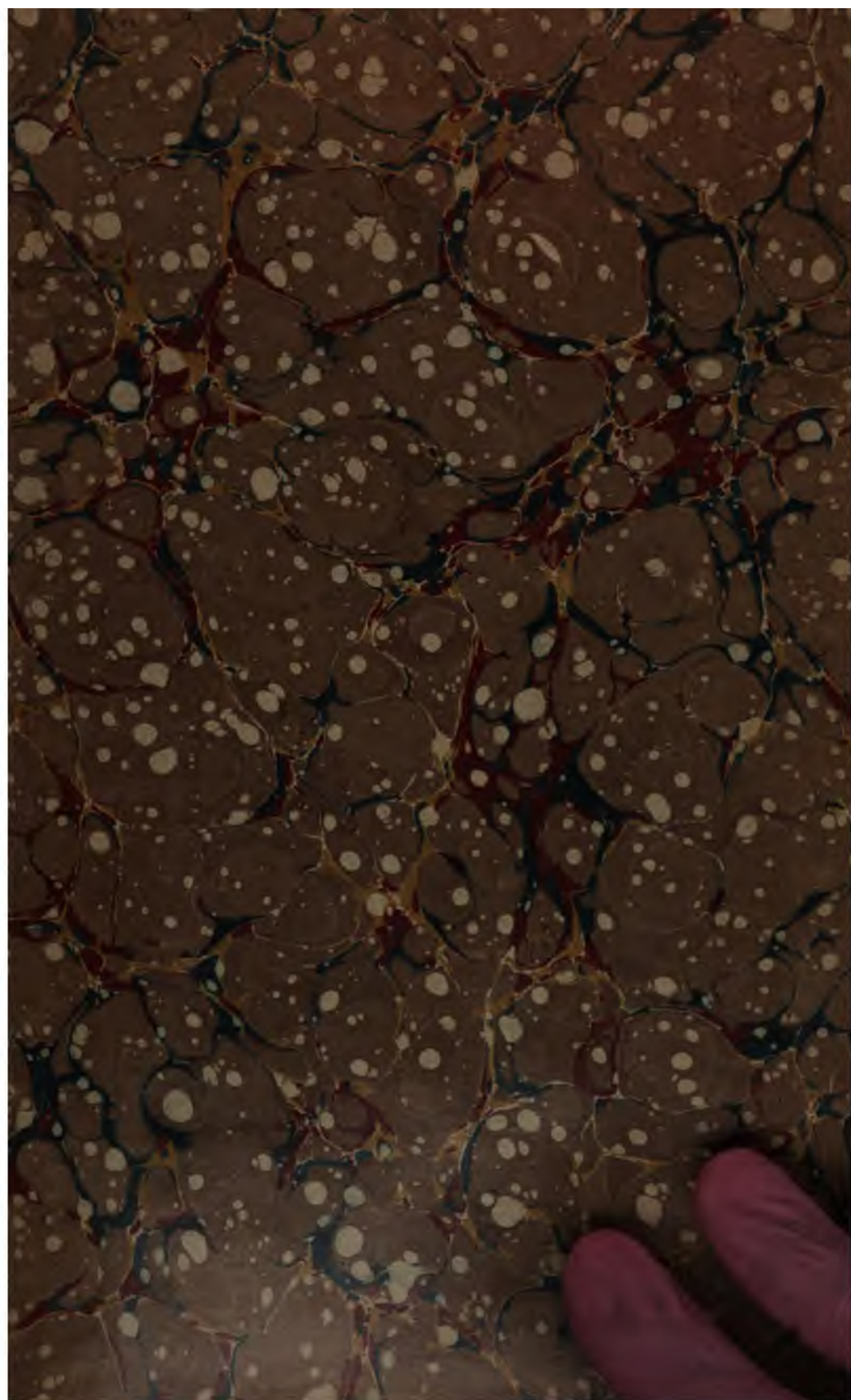
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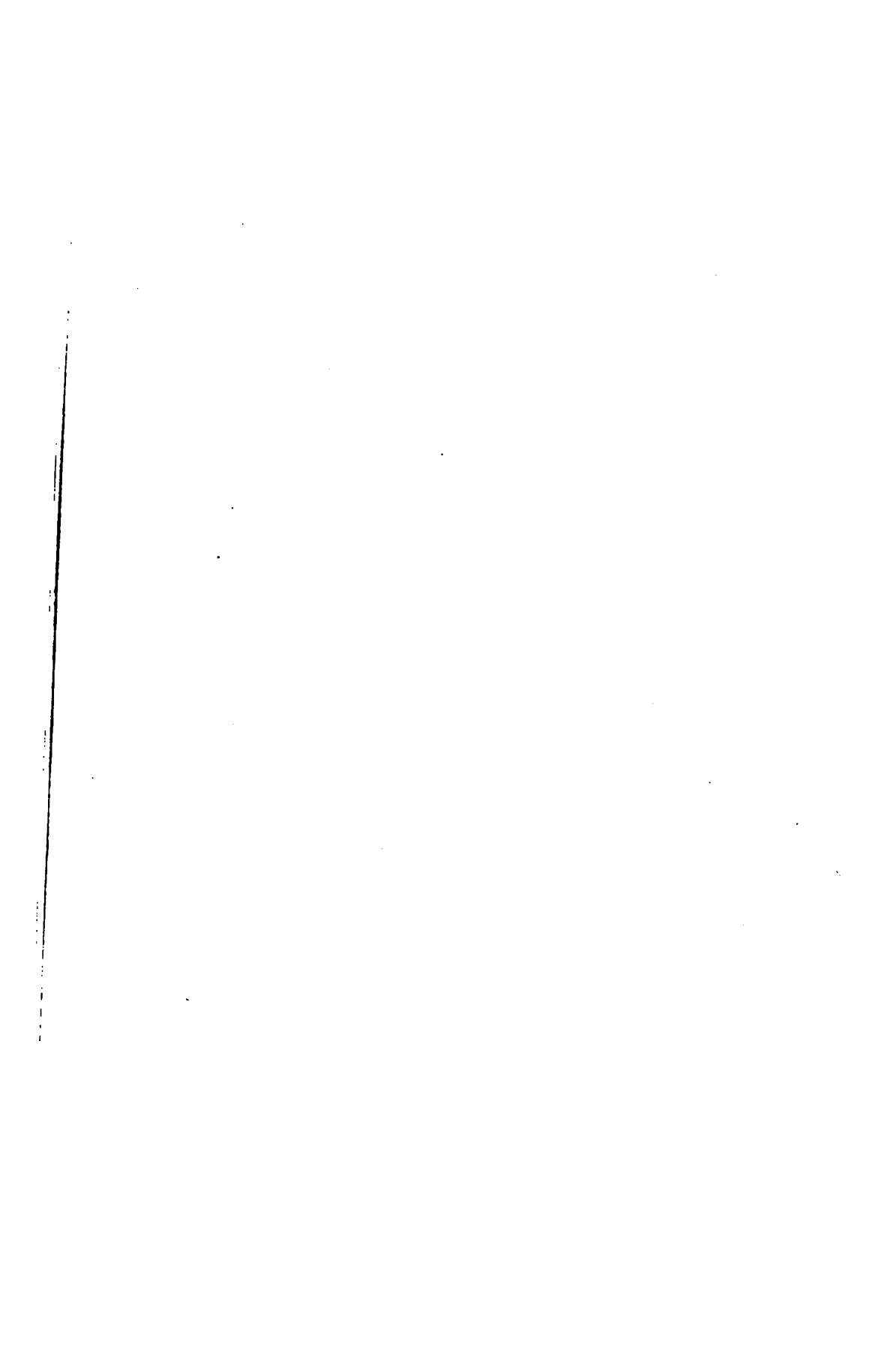
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NO. I.

THE NATURE AND ACTION OF THE THROMBOPLASTIC
(ZYMOPLASTIC) SUBSTANCE OF THE TISSUES.

By W. H. HOWELL.

[*From the Physiological Laboratory of the Johns Hopkins University.*]

IT has long been known that the coagulation of blood is hastened by contact with the tissues. This fact has been demonstrated by Arthus¹ and others for the mammalian blood, but the accelerating effect of the tissues is shown most strikingly in the case of those bloods whose normal, so to speak, intrinsic coagulation occurs very slowly. Such bloods are especially the blood of the birds when removed carefully from the blood vessels so as not to come into contact with the tissues, and the so-called peptonized blood of the dog. Bloods of this kind may be made to clot promptly by the addition of saline or aqueous extracts of various tissues, thymus gland, muscle, brain, testis, etc. The extractible substance or substances in the tissues which facilitate the process of clotting, which indeed under normal conditions are essential to the prompt clotting of the blood, have been designated as zymoplastic (Schmidt) or thromboplastic (Nolf) substances, although other names of a more specific character have been suggested. The explanation of the manner of action of these substances has varied naturally with the views adopted regarding the nature of the processes of coagulation. According to some authors (Pekelharing, Loeb, *et al.*), they are substances identical with or similar in action to the fibrin ferment (thrombin). According to Arthus, they are substances of an unknown nature which accelerate the

¹ ARTHUS: *Journal de physiologie et de pathologie*, 1902, iv, p. 281.

formation of thrombin by action upon the leucocytes. Bang² has suggested a somewhat similar view, namely, that the thromboplastic substances are lipoids which stimulate or activate the tissue cells to produce thrombokinase. Both of these views which attribute to the thromboplastic substance a stimulating action upon formed elements would seem to be contradicted conclusively by Hewlett's experiments. This author³ showed that the cell-free plasma of bird's blood is made to clot by the addition of tissue extracts, and a similar result may be demonstrated easily for cell-free peptone plasmas. Schmidt explained the action of the thromboplastic material on the view that they cause the formation of thrombin from prothrombin by a process of splitting, or, as we might say now in more general terms, by a process of activation. An essentially similar hypothesis, although expressed in different terms, has been advanced by Morawitz⁴ and by Fuld and Spiro.⁵ They assume that the thromboplastic substance is of the nature of an activator or co-ferment which is essential to the conversion of prothrombin (thrombogen) to active thrombin. Morawitz designated the substance as a kinase (thrombokinase), while Fuld and Spiro described it under the name cytozym. In Nolf's⁶ somewhat intricate theory of coagulation the favoring action of thromboplastic agents is explained on the view that they act as centres of precipitation in the colloidal interaction between fibrinogen, thrombogen, and thrombozym. In contrast to these theories the author⁷ has furnished evidence to show that the thromboplastic material in tissue extracts facilitates clotting only in those plasmas in which antithrombin is present and that it acts by neutralizing the antithrombin.

It is easily proved and is perhaps generally admitted that avian blood and the blood of a peptonized dog owe their relative incoagulability to antithrombin, the presence of which can be detected and he amount estimated relatively by the use of test solutions contain-

² BANG: *Ergebnisse der Physiologie*, 1909, viii, p. 476.

³ HEWLETT: *Archiv für experimentelle Pathologie und Pharmakologie*, xlix, p. 307.

⁴ MORAWITZ: *HOFMEISTER'S Beiträge zur chemische Physiologie und Pathologie*, 1904, iv, p. 381; also *Ergebnisse der Physiologie*, 1905, iv, p. 405.

⁵ FULD and SPIRO: *Ibid.*, 1904, v, p. 174.

⁶ NOLF: *Archives internationales de physiologie*, 1908, vi, pp. 1 and 115; also *Revue générale des sciences pures et appliquées*, 1909, July 15.

⁷ HOWELL: *this Journal*, 1911, xxix, p. 187.

ing definite amounts of thrombin and fibrinogen. These bloods can be made to clot by the direct addition of thrombin solutions provided enough is added to overcome the excess of antithrombin present. The older statement of some authors that peptonized blood does not clot upon the addition of fibrin ferment (thrombin) is entirely erroneous and due to the fact that in their experiments too little thrombin was used. On the other hand, peptonized plasma is clotted very readily by the addition of tissue extracts. In this case what takes place is that the thromboplastic substance in the extracts neutralizes the antithrombin, and thus permits the thrombin already present in the blood to effect its normal action upon the fibrinogen. The author⁸ has shown that antithrombin in small amounts is a constant constituent of mammalian blood, and that the facilitation of the clotting of this blood caused by tissue extracts, or by simple contact with the tissues, is due to the fact that the antithrombin is neutralized by the thromboplastic substance in the tissues. Further evidence in favor of this explanation of the action of thromboplastic substance is furnished in this paper.

Much uncertainty has prevailed in regard to the chemical nature of the thromboplastic substance. It has been especially confusing to find that some authors have stated that this substance is water-soluble only and that its activity is destroyed by heating, while others have claimed that it may be extracted from the tissues with alcohol or ether and that its action is not destroyed by boiling. It would seem at first, from these and other contradictory statements, that there must be a variety of thromboplastic substances. As will be shown, however, in this paper, these contradictory statements may be reconciled, and there is every reason to believe that the thromboplastic substance is essentially the same in all tissues and acts in all cases in the same way.

Thromboplastic solutions are made usually by extracting a minced tissue with water or a saline solution. The extract should be freed from suspended particles by repeated filtration or by centrifugalization. As a rule, the extracts are very opalescent, but they may be obtained nearly clear by repeated filtration while still very active. The most successful perhaps of the efforts made to isolate further the active substance in these extracts is that reported by Wooldridge.

⁸ HOWELL: *Loc. cit.*

Wooldridge himself did not accept the usual theory of coagulation, that is to say, a reaction between fibrinogen and thrombin, and he did not make use of the term thromboplastic or zymoplastic substance. There can be little doubt, however, that his A-fibrinogen of the blood, or his tissue fibrinogen obtained from other tissues, is identical with what other authors have designated as thromboplastic or zymoplastic substance. In 1886⁹ he prepared this substance from the thymus gland by the following method. Calf's thymus was extracted with water and the extract was centrifugalized. The solution was then made strongly acid with acetic acid, and the voluminous precipitate obtained was washed with water and then dissolved in a dilute solution of sodium carbonate. He showed that such solutions cause intravascular clotting when injected into the circulation, that they have no direct thrombin action as tested upon magnesium-sulphate plasmas, and that the active constituent may be extracted from thymus tissue with alcohol and ether. In recent years the same method substantially has been described by Horneffer¹⁰ and Battelli¹¹ for the preparation of what they designate as pure thrombokinas. The substance precipitated by the acetic acid is evidently a protein, but whether or not its undoubted activity in facilitating the process of clotting is due to the protein or to some accompanying substance was not determined. Schmidt long ago claimed that an active zymoplastic substance can be extracted from the tissues with alcohol and ether, and he suggested that possibly this substance is lecithin. Wooldridge also found that alcohol-ether extracts of red or white blood corpuscles or of other tissues yielded a substance corresponding with what was then known as lecithin, which had the same action in clotting peptone plasmas as aqueous extracts of the tissues. He stated, however, that lecithin prepared from the yolk of egg has no such action. This curious difference indicated that the active substance in the alcohol-ether extract of the tissues is either something else than lecithin, or that the lecithin of the tissues varies in some way from the stored lecithin of the egg yolk. While Wooldridge did not identify the active material in thromboplastic solutions as lecithin, since the whole conception of thromboplastic activity was excluded from his theory,

⁹ WOOLDRIDGE: *Archiv für Physiologie*, 1886, p. 397.

¹⁰ HORNEFFER: *Thèse de Genève*, 1908.

¹¹ BATTELLI: *Comptes rendus de la Société de Biologie*, 1910, May 7.

yet, as a matter of fact, with our present knowledge we must interpret his results, together with those of Schmidt, as pointing to the belief that a lecithin is the active constituent in thromboplastic extracts. The present paper gives an account of experiments and observations the end result of which has been to strengthen this conclusion and to reconcile it with the apparently contradictory results reported by other authors.

PREPARATION OF THE PEPTONE PLASMA.

In experiments upon the thromboplastic activity of tissue extracts it is necessary above all things to have a suitable solution upon which the facilitating action of these extracts may be tested. The centrifugalized plasma of a peptonized dog furnishes such a solution, but, as is well known, it is somewhat difficult to prepare such plasmas so that they shall have a constant coagulability. According to the dose and the character of the peptone used and the time after peptonization at which the blood is drawn the plasma may contain little or much antithrombin and will show varying degrees of coagulability. Even when all the conditions are kept as constant as possible, the effect of the injection, so far as the coagulability of the drawn blood is concerned, may vary with the animal used. This difficulty has been avoided in these experiments by preparing a large amount of clear peptone plasma from one dog and drying it down to make a stock material. To do this the plasma was evaporated to dryness in watch crystals, each containing 2 c.c. of the plasma, placed in a current of warm air but with precautions to keep the temperature below 40° C. The watch crystals containing the dried plasma were kept in a desiccator, and when a specimen of peptone plasma was needed the material in one of the crystals was rubbed up with 4 c.c. of a 0.9 per cent solution of sodium chloride and filtered. The plasma thus obtained may be used directly or, in order to increase its coagulability, and therefore its sensitiveness as a reagent for the detection of thromboplastic substance, it may be diluted with an equal volume of water. The peptone plasma originally used was obtained by injecting a solution of Witte's peptone of known efficiency into a dog in the proportion of 0.4 to 0.5 gm. to each kilogram of weight of the animal. The dog was anesthetized with morphia and ether, and the whole amount of peptone

used was forced quickly into the circulation by injection into the femoral artery under pressure. In order that the plasma may be free from fat it is desirable that the animal should have fasted for twenty-four hours. Twenty minutes after the injection the animal was bled to death, the blood was centrifugalized at once, and the clear plasma was evaporated to dryness in watch crystals, as described above. In this way material was obtained for a series of experiments whose coagulability toward thromboplastic solutions was quite uniform. It may be stated that the peptonized plasma used did not clot spontaneously in twenty-four hours, even when diluted with two to three times its volume of water. The addition of water to the saline solution of dried peptone plasma greatly increases its sensitiveness toward thromboplastic solutions, as may be illustrated by the following experiment. The thromboplastic solution used in this case was a saline extract of brain made by treating 1 gm. of dried brain with 100 c.c. of saline solution (0.9 per cent sodium chloride) and centrifugalizing.

1. Peptone plasma, five drops; water, twenty drops. No clot in twenty-four hours.
2. Peptone plasma, five drops; brain extract, five drops. No clot in twenty-four hours.
3. Peptone plasma, five drops; water, five drops; brain extract, five drops. Solid clot in seven minutes.
4. Peptone plasma, five drops; water, ten drops; brain extract, five drops. Solid clot in five minutes.

This result was constant and agrees with the observation previously reported by the author upon the effect of diluting the slowly coagulating centrifugalized plasma of the terrapin with water and with saline solution respectively. It would seem that, in plasmas containing sufficient antithrombin to retard or to prevent spontaneous coagulation, dilution with approximately isotonic solutions of sodium chloride does not alter the coagulability of the plasma, while dilution with water increases the rapidity with which spontaneous coagulation occurs in solutions containing little antithrombin, or increases greatly the sensitiveness toward the coagulating effect of thromboplastic solutions in plasmas containing much antithrombin. Dilution with water, in other words, weakens the action of antithrombin. A satisfactory explanation of this fact cannot be given until further experiments are made.

PREPARATION OF THE THROMBOPLASTIC SOLUTIONS.

In preparing thromboplastic solutions from the various tissues it was found most convenient to start with the dried tissue. The organ selected, usually the brain or the thymus gland, was washed as free as possible from blood and was then ground to a thin pulp in a mortar. This material was spread in thin layers on glass plates and was dried at a low temperature in a current of warm air. The dry material was kept in a desiccator until needed.

Ether extracts. — The thromboplastic material may be obtained from the dried tissue by extraction with alcohol, ether, or chloroform. Ether was found to be most satisfactory, and the results obtained with this solvent will be described in detail. The dried tissue, preferably the brain, was shaken with a large volume of ether and allowed to stand for some hours. The red-colored solution was filtered once or twice, until clear, and was then evaporated to dryness in a current of warm air. The soft oily reddish residue when treated with water gave a turbid solution which had a marked thromboplastic action on peptone plasma. This residue was further treated as follows. It was first extracted several times with large volumes of cold acetone and finally with hot acetone. This solvent removed the fats, cholesterol, and cholesterol esters. The first acetone extract was rich in cholesterol and cholesterol esters, and when evaporated to dryness and taken up with water in the form of an emulsion showed no thromboplastic effect at all upon peptone plasma. The later acetone extracts and particularly the hot acetone extract yielded a small residue which upon treatment with water gave a solution that exhibited a feeble but distinct thromboplastic action on peptone plasma. The results of the acetone extraction show that the thromboplastic substance is not cholesterol or a cholesterol ester. Some previous observations had led me to suspect that the active substance might be one of the cholesterol esters, but the procedure here described proves that this suspicion was not correct, and this conclusion was verified by experiments with cholesteryl oleate, prepared synthetically, which showed that this substance in aqueous suspension is entirely inactive toward peptone plasmas. It is evident, however, that the active substance is slightly soluble in the acetone, especially in the hot acetone.

The residue left after the treatment with acetone had a waxy con-

sistency and was strongly thromboplastic in aqueous solution. It was treated next with a large bulk of cold alcohol. The alcoholic extract evaporated to dryness yielded a considerable residue containing a large proportion of lecithin. This residue treated with water gave an opalescent solution which had a distinct thromboplastic action, while the portion not soluble in cold alcohol when treated with water gave likewise an opalescent solution possessing marked thromboplastic action. The portion soluble in cold alcohol was evaporated to dryness, the residue was dissolved in ether, and the latter solution was precipitated with acetone. This process was repeated, and the second acetone precipitate was dissolved with some difficulty in warm alcohol; evaporated to dryness and treated with water, solutions were obtained showing thromboplastic action. It is evident that the active substance is soluble to some extent in cold alcohol, but its solubility in this solvent must be small, since a relatively large volume of alcohol was used and most of the active substance remained behind. The material remaining after extraction with cold alcohol was next treated repeatedly with large volumes of boiling alcohol. Each such treatment dissolved a part of the reddish residue, and after four or five extractions the small residue finally remaining ceased to exhibit thromboplastic action when treated with water and tested upon a peptone plasma. Each of the extracts with hot alcohol, especially the first one, gave on evaporation a reddish waxy deposit which when rubbed up with water dissolved easily to form an opalescent solution possessing marked thromboplastic action. It would appear from these results that the active substance is soluble with difficulty in hot alcohol. The residues obtained by evaporating the cold and hot alcoholic extracts consisted mainly of lecithin and related phosphatids. They contained phosphorus in considerable amounts and gave the usual myelin figures upon treatment with water.

The question of most interest is to determine whether the thromboplastic substance is lecithin itself or one of the related phosphatids, or whether perhaps this property is possessed by all the substances belonging to this group. Owing to the confusion that exists in regard to the chemistry of these bodies, it is not possible to answer this question with entire satisfaction. The chemical individuality of the several phosphatids described is not well established, and, as is well known, there is no satisfactory method of obtaining pure lecithin from the

brain or other tissues. The results of the numerous efforts made to isolate the active thromboplastic substance as found in the ether extracts of brain or thymus have led me to believe that this substance is not lecithin as usually defined, but rather the related unsaturated phosphatid, kephalin, or else, although this suggestion seems highly improbable, some unknown substance which adheres to the kephalin fraction of the phosphatid material. The reasons for this conclusion rest upon such reactions as the following: an ether extract of the dried brain was evaporated to dryness, and the residue was extracted thoroughly with acetone to remove the fats, cholesterin, and cholesterin esters. The residue remaining after the acetone extraction was dissolved in ether and precipitated by an excess of alcohol. The precipitate was separated by centrifugalizing, was dissolved in ether, and again precipitated by excess of alcohol. Since lecithin as usually described is not precipitated from its ethereal solutions by addition of alcohol, the final precipitate obtained by this treatment might be expected to consist of kephalin or of kephalin and myelin. This material in aqueous solution exhibited thromboplastic action. When treated with ether, it dissolved readily, although the solution was somewhat turbid. The ethereal solution was evaporated to dryness, and the residue was treated with a large bulk of hot alcohol. Only a portion of the residue dissolved, as would be expected from the known small solubility of kephalin even in hot alcohol. The alcoholic solution while hot was precipitated by the addition of a hot alcoholic solution of lead acetate with ammonia. An abundant precipitate was obtained, which when cold dissolved readily in ether. The ready solubility of this precipitate in ether as well as the rapid solution of the previous alcoholic precipitate in ether would indicate that no myelin was present, while, on the other hand, the solubility relations serve to identify the phosphatid as a kephalin. We must believe, therefore, that the thromboplastic action of brain and thymus tissue, and probably of all tissues, is due in part at least to the presence of a phosphatid corresponding in its properties to kephalin.

Whether or not lecithin possesses a similar property is difficult to determine. If we accept the view that brain lecithin is identical chemically with the lecithin found in the yolk of the egg, then the facts indicate that this phosphatid is without thromboplastic action. Woolbridge, as stated above, called attention to the fact that lecithin pre-

pared from the egg has no effect upon the clotting of peptone plasma, while that obtained from the tissues accelerates the clotting after the manner of tissue extracts, and in this connection it is suggestive that Koch in his investigations came to the conclusion that kephalin, as described by him, does not occur in the egg, "which consists mostly of stored material," but can be obtained only from the substance of living cells. As will be shown below, this latter statement is too sweeping, but it is very easy to show that lecithin as prepared from the egg, is entirely lacking in thromboplastic action. In demonstrating this fact I have used specimens of lecithin freshly prepared from the egg, as also several specimens which I owe to the kindness of Dr. Kyes of the University of Chicago. These latter specimens were labelled egg lecithin, Agfa, egg lecithin, Blattmann, and egg lecithin, Riedel. Each of these preparations when treated with water gave a turbid solution or emulsion, which, when added, in equal volume, to solutions of my dried peptone plasma, gave no indications of clotting after a stand of twenty-four hours. Parallel preparations made with the above-described extracts (kephalin) from the brain or thymus caused clotting in twenty to thirty minutes. A specimen of plant lecithin (Blattmann) and of so-called brain lecithin, also furnished by Dr. Kyes, possessed a distinct thromboplastic action when used in aqueous solutions. Similar negative results were obtained from the egg lecithins after they had been purified by acetone precipitation from ethereal solutions. In making fresh preparations of lecithin from the egg the same procedure was followed as in the case of the brain; that is to say, the yolks were rubbed in a mortar, spread in thin layers on glass plates, and dried in a current of warm air. The dried material was powdered and extracted with ether. The yellow ethereal solution was evaporated to dryness and extracted thoroughly with acetone. The waxy material remaining consisted chiefly of lecithin. When treated with water, it gave an opalescent solution which, tested upon specimens of peptone plasma, showed a very feeble indication of thromboplastic action. After a number of hours there might be formed an imperfect membranous clot at the bottom of the tubes. The waxy residue was further treated with cold alcohol; most of it dissolved, and the small portion remaining was only partly soluble or difficultly soluble in hot alcohol. The portion soluble in cold alcohol and the insoluble portion were treated separately for their thromboplastic action. The

former, which consisted of the lecithin proper, when brought into aqueous solution, gave wholly negative results with the peptone plasma. The portion insoluble in cold alcohol was further purified by solution in ether and precipitation with acetone. In aqueous solution it had a distinct thromboplastic effect upon the peptone plasma. It would seem from these observations that there is contained in the egg yolk a small portion of phosphatid material possessing the solubility relations of kephalin, and this material has a thromboplastic action, while the egg lecithin is entirely devoid of this property. Stern and Thierfelder¹² also report that they have prepared from egg yolk a kephalin-like body which is only slightly soluble in hot alcohol.

It was stated above that a specimen of plant lecithin, unlike the egg lecithins, showed distinct thromboplastic action. Closer examination showed, however, that the active substance in this specimen was again a kephalin-like body. The specimen was dissolved in ether and the solution was precipitated by acetone. The precipitate was treated successively with cold and hot alcohol. It was found that a portion of the precipitate was soluble in cold alcohol, a portion was soluble in hot alcohol, and a portion was insoluble or soluble with great difficulty in hot alcohol. The three portions were tested separately for their thromboplastic action. This action was quite distinct only for the part soluble in hot alcohol but insoluble in cold alcohol. Since the lecithin proper was found chiefly at least in the portion soluble in the cold alcohol, the negative reaction from it would again indicate that the lecithin does not possess thromboplastic action.

Although it is perfectly clear that egg lecithin does not possess thromboplastic properties, it still remains uncertain whether or not this statement can be made positively for the brain lecithin. The kephalin of the brain tissue is undoubtedly thromboplastic, but my experience indicates that the active phosphatid in the brain is more easily soluble in alcohol than would be expected from current descriptions of the properties of kephalin. On this latter point, however, there seems to be considerable difference of opinion, the kephalin in fact being an insufficiently characterized body. The method of separation between lecithin and kephalin by treatment with cold alcohol is unsatisfactory, as appears from results such as the following. The

¹² STERN and THIERFELDER: *Zeitschrift für physiologische Chemie*, 1907, liii, p. 378.

dried brain was extracted with ether, the ethereal solution was evaporated to dryness, and the residue was extracted thoroughly with acetone. The portion remaining was redissolved in ether and precipitated by the addition of alcohol. Examination showed that thromboplastic substance was present in the precipitate, which contained kephalin, as well as in the filtrate, which contained the lecithin. The filtrate was evaporated to dryness, the residue was dissolved in ether and precipitated by addition of acetone. The precipitate obtained when treated with cold alcohol dissolved for the most part, and the material in solution on examination showed distinct thromboplastic action. The alcoholic solution was evaporated to dryness, and the residue was taken up with hot acetic ether. On cooling, the solution gave a white deposit which showed thromboplastic action. This material consisted for the most part certainly of lecithin, but it remains possible that enough kephalin or related phosphatid passed into solution in the alcohol to explain the thromboplastic effect. Under the circumstances it is not possible to state definitely whether or not brain lecithin has thromboplastic action, but it would seem probable that it has not, considering the clearly negative results yielded by egg lecithin.

Saline and aqueous extracts. — In making saline or aqueous extracts 1 gm. of the dried tissue was rubbed up with 100 c.c. of water or of a 0.9 per cent solution of sodium chloride. The very turbid solution obtained was centrifugalized or filtered until as clear as possible. In the case of the brain extracts made with water centrifugalization at 3000 gives an opalescent solution, and the sediment, if again treated with water and centrifugalized, yields also a turbid solution showing thromboplastic action. If the process of treating the sediment with water and centrifugalizing is continued, solutions of decreasing opacity and decreasing thromboplastic action are obtained. It is evident that in the dried brain tissue the active material is only slightly soluble in water, but by repeated extraction it may be dissolved out completely. The solutions, particularly the later clearer ones which are entirely free from suspended particles, give no visible coagulum on boiling and none of the usual protein reactions. On the contrary, the aqueous and saline extracts of the dried thymus gland and the saline extracts of the dried brain contain a protein material with which the active thromboplastic substance is combined. This protein is more abundant in the extracts from the thymus than in those from the brain. As stated

by Wooldridge, the addition of acetic acid throws down a protein material which may be redissolved in dilute solutions of sodium carbonate (thymus) and which has a strong thromboplastic action. When the saline extracts of the thymus are heated to 56° to 60° C., an abundant coagulum forms containing all the protein material. The filtrate from this precipitate has lost entirely its thromboplastic property. The most interesting fact in view of the preceding evidence in regard to the phosphatid nature of the thromboplastic substance is that when either the acetic acid precipitate or the heat coagulum is extracted with alcohol-ether an active thromboplastic substance is found in the extract. The ether or alcohol-ether extract evaporated to dryness leaves a residue which when rubbed up with water gives an opalescent solution possessing thromboplastic properties. This latter solution may be boiled without affecting its thromboplastic activity. We must conclude that in the brain and thymus, and no doubt in the tissues generally, the thromboplastic phosphatid material is combined with a tissue protein having a low temperature of heat coagulation. This protein corresponds with what Wooldridge called tissue fibrinogen, and as he suggests it may be classified in the lecithin protein group. Instead of the term tissue fibrinogen it would be better to describe this protein simply as tissue globulin, since it does not possess any direct relation to the formation of fibrin. It is not precipitated from its solutions by saturation with sodium chloride, but is thrown down by half saturation with ammonium sulphate. The precipitate in the latter case when redissolved in water exhibits the thromboplastic action of the original extract. It is evident also from the above facts that the destruction of thromboplastic activity by heating in saline and aqueous extracts of the tissues is due simply to the coagulation of this protein which carries down with it the active phosphatid material; the latter is not injured by the boiling. The contradiction between those who claim that the thromboplastic substance is thermolabile and those who insist that it is thermostable may therefore be reconciled.

The former are correct so far as the aqueous and saline extracts of the tissues are concerned, and the latter are correct in the case of the alcohol-ether extracts. The phosphatid body in aqueous solution is entirely thermostable, but in combination with tissue globulin it is precipitated out of solution by heat or other agencies which cause a coagulation of the protein, although itself not injured by the process, since it may be extracted readily from the coagulum by ether.

THE ACTION OF THE THROMBOPLASTIC SUBSTANCE.

That the thromboplastic action of the phosphatid (kephalin) solutions is due to a neutralization of antithrombin can be shown by direct experiments of the following character. In one set of experiments the thromboplastic solution was prepared as follows. The dried brain was extracted with ether. The ethereal solution was evaporated to dryness. The residue was extracted thoroughly with acetone to remove the cholesterol and fats and again dried at a low temperature. It was then dissolved in ether, and the ethereal solution was precipitated by the addition of alcohol. The precipitate and filtrate both exhibited thromboplastic action. The experiments were carried out with the filtrate, which was evaporated to dryness and then treated with water. The action of the opalescent solution obtained was tested upon hirudin and upon the antithrombin contained in peptone plasma. The hirudin solution was made by dissolving 0.1 gm. of commercial hirudin in 200 c.c. of a 0.9 per cent solution of sodium chloride, giving an approximate concentration of 1 to 20,000. The antithrombin of the peptone plasma was prepared by dissolving one of the watch crystals containing the dried plasma in 4 c.c. of a 0.9 per cent solution of sodium chloride, filtering and heating to 60° C. to precipitate the fibrinogen. The filtrate from this precipitate contained the antithrombin.

The idea of the experiments was to mix certain amounts of the thromboplastic solution with certain amounts of the hirudin or antithrombin solution and allow these mixtures to stand for a specified time, in one case for thirty minutes and in another for five hours. The action of these mixtures was then compared with similar mixtures in which the thromboplastic solution had been replaced by water and which had been allowed to stand for the same time. The comparisons were made by adding the mixtures in suitable amounts to a series of thrombin and plasma mixtures whose time of coagulation was known (three to eight minutes) and in which the amounts of thrombin were varied.

The plasma was used in place of a solution of fibrinogen and consisted of oxalated and centrifugalized dog's blood from which the excess of oxalate had been removed by dialysis. The clear plasma was then dried at low temperature in watch crystals and kept as a stock material of uniform properties. The results of the first series of observations may be stated as follows:

EXPERIMENTS WITH HIRUDIN. STAND OF THIRTY MINUTES.

I. Hirudin solution (1-20000) plus equal volume of water (Mixture A).

II. Hirudin solution (1-20000) plus equal volume of thromboplastic solution (Mixture B).

Control series.—1. Mixture A, one drop; thrombin solution, three drops. Allowed to stand fifteen minutes, then added thirteen drops of plasma. No clot after twenty-four hours.

2. Mixture A, one drop; thrombin solution, four drops. Allowed to stand fifteen minutes, then thirteen drops of plasma. No clot after twenty-four hours.

3. Mixture A, one drop; thrombin solution, five drops. Allowed to stand fifteen minutes, then thirteen drops of plasma. Clotted partially in five hours thirty minutes.

4. Mixture A, one drop; thrombin solution, six drops. Allowed to stand fifteen minutes, then thirteen drops of plasma. Clotted in thirty minutes.

Series with thromboplastic solution.—1. Mixture B, one drop; thrombin solution, three drops. Allowed to stand fifteen minutes, then thirteen drops of plasma. Clotted in fifty-five minutes.

2. Mixture B, one drop; thrombin solution, four drops. Allowed to stand fifteen minutes, then thirteen drops of plasma. Clotted in thirty to thirty-five minutes.

3. Mixture B, one drop; thrombin solution, five drops. Allowed to stand fifteen minutes, then thirteen drops of plasma. Clotted in ten minutes.

4. Mixture B, one drop; thrombin solution, six drops. Allowed to stand fifteen minutes, then thirteen drops of plasma. Clotted in five to ten minutes.

EXPERIMENTS WITH ANTITHROMBIN FROM PEPTONE PLASMA. STAND OF THIRTY MINUTES.

I. Antithrombin solution plus equal volume of water (Mixture A).

II. Antithrombin solution plus equal volume of thromboplastic solution (Mixture B).

Control series. — 1. Mixture A, one drop; thrombin solution, three drops. Allowed to stand fifteen minutes, then thirteen drops of plasma. Small membranous clot after twenty-four hours.

2. Mixture A, one drop; thrombin solution, four drops. Allowed to stand fifteen minutes, then thirteen drops of plasma. Beginning clot after six hours, fully clotted next morning (twenty hours).
3. Mixture A, one drop; thrombin solution, five drops. Allowed to stand fifteen minutes, then thirteen drops of plasma. Clotted between one hour fifty-five minutes and two hours five minutes.
4. Mixture A, one drop; thrombin solution, six drops. Allowed to stand fifteen minutes, then thirteen drops of plasma. Beginning clot in sixty-five minutes, complete at one hour twenty minutes.

Series with thromboplastic solution. — 1. Mixture B, one drop; thrombin solution, three drops. Allowed to stand fifteen minutes, then thirteen drops of plasma. Clotted in sixty minutes.

2. Mixture B, one drop; thrombin solution, four drops. Allowed to stand fifteen minutes, then thirteen drops of plasma. Clotted in thirty-five minutes.
3. Mixture B, one drop; thrombin solution, five drops. Allowed to stand fifteen minutes, then thirteen drops of plasma. Clotted in fifteen minutes.
4. Mixture B, one drop; thrombin solution, six drops. Allowed to stand fifteen minutes, then thirteen drops of plasma. Clotted in ten to fifteen minutes.

Exactly similar results were obtained in a series made with the same solutions and in which the only variation introduced was that the mixtures of antithrombin and thromboplastic substance were allowed to stand five hours instead of thirty minutes before being tested upon the solutions of thrombin and plasma. The results of the second series showed that the neutralizing action of the kephalin solutions was as complete after thirty minutes as at the end of five hours. In a third series of experiments the thromboplastic solution was prepared from the residue of an ethereal extract of the brain after previous extraction with cold alcohol alone. Its action was tested upon the antithrombin of a peptone plasma by two different procedures: first, a solution in saline of the unheated peptone plasma was mixed with an equal volume of the thromboplastic solution, and after standing ten minutes but before clotting had occurred it was heated to 60° C. to precipitate

the fibrinogen. The filtrate (Mixture B) was tested for its anti-thrombic power in comparison with a control mixture (Mixture A) treated in the same way except that water was used in place of the thromboplastic solution.

I. Control with water (Mixture A).

1. Mixture A, one drop; thrombin solution, three drops. Allowed to stand fifteen minutes, then plasma thirteen drops. No clot at end of two hours; found clotted next morning (twenty hours).
2. Mixture A, one drop; thrombin solution, four drops. Allowed to stand fifteen minutes, then plasma thirteen drops. Clotted in one hundred minutes.
3. Mixture A, one drop; thrombin solution, five drops. Allowed to stand fifteen minutes, then plasma thirteen drops. Clotted in twenty-six minutes.
4. Mixture A, one drop; thrombin solution, six drops. Allowed to stand fifteen minutes, then plasma thirteen drops. Clotted in ten minutes.

II. With thromboplastic solution (Mixture B).

1. Mixture B, one drop; thrombin solution, three drops. Allowed to stand fifteen minutes, then plasma thirteen drops. Clotted in sixty-five to seventy minutes.
2. Mixture B, one drop; thrombin solution, four drops. Allowed to stand fifteen minutes, then plasma thirteen drops. Clotted in thirty-five minutes.
3. Mixture B, one drop; thrombin solution, five drops. Allowed to stand fifteen minutes, then plasma thirteen drops. Clotted in fifteen minutes.
4. Mixture B, one drop; thrombin solution, six drops. Allowed to stand fifteen minutes, then plasma thirteen drops. Clotted in ten minutes.

In the second procedure the saline solution of peptone plasma was heated to 60°C. to precipitate the fibrinogen, and the filtrate containing the antithrombin was divided into two parts. The first portion was mixed with an equal volume of thromboplastic solution (Mixture B) and the second with an equal volume of water (Mixture A). The two

mixtures were allowed to stand for twenty-five minutes and were then tested for their antithrombic power.

I. The control mixture (Mixture A).

1. Mixture A, one drop; thrombin solution, three drops. Allowed to stand fifteen minutes, then plasma thirteen drops. No sign of clotting after two hours; imperfect clot after twenty-four hours.
2. Mixture A, one drop; thrombin solution, four drops. Allowed to stand fifteen minutes, then plasma thirteen drops. Beginning clot in two hours; found clotted in morning (twenty hours).
3. Mixture A, one drop; thrombin solution, five drops. Allowed to stand fifteen minutes, then plasma thirteen drops. Beginning clot at fifty minutes, nearly complete at sixty-five minutes.
4. Mixture A, one drop; thrombin solution, six drops. Allowed to stand fifteen minutes, then plasma thirteen drops. Clotted in twenty minutes.

II. The thromboplastic mixture (Mixture B).

1. Mixture B, one drop; thrombin solution, three drops. Allowed to stand fifteen minutes, then plasma thirteen drops. Beginning clot at seventy minutes, solid clot at one hundred five minutes.
2. Mixture B, one drop; thrombin solution, four drops. Allowed to stand fifteen minutes, then plasma thirteen drops. Solid clot between forty and fifty-five minutes.
3. Mixture B, one drop; thrombin solution, five drops. Allowed to stand fifteen minutes, then plasma thirteen drops. Clotted between twenty-five and thirty minutes.
4. Mixture B, one drop; thrombin solution, six drops. Allowed to stand fifteen minutes, then plasma thirteen drops. Clotted at fifteen minutes.

These experiments all seem to give direct and conclusive proof that the thromboplastic solutions of brain phosphatid (kephalin) favor coagulation, in liquids containing antithrombin, by neutralizing the action of the antithrombin. So far as the author can see the only objection that can be made to this conclusion rests upon the hypothesis that the thromboplastic substance instead of neutralizing the antithrombin may have caused the production of more thrombin, by activation of prothrombin, in the plasma used for the coagulation test.

This objection is made invalid by the fact that the thromboplastic solutions added alone to the plasma used caused no coagulation, hence they had no power to convert prothrombin to thrombin. The further intrinsically improbable suggestion that the thromboplastic substance in some way may have augmented the supply of prothrombin in the plasma is also irrelevant. The plasmas used for the coagulation tests were oxalated plasmas, subsequently dialyzed to remove excess of oxalate, hence they were calcium free, and granting that in some way their supply of prothrombin may have been increased there was no chance for this substance to become activated to thrombin in a calcium-free mixture. It would have been possible of course in these experiments to have used a solution of fibrinogen instead of the oxalated plasma, but this did not seem to be necessary, and on the other hand it was very convenient to use the oxalated plasma, since, as described above, it could be kept on hand as a stock material of uniform composition. It is not possible to keep fibrinogen solutions by drying them down, as in the case of the plasma, nor is it possible to prepare from time to time fresh fibrinogen solutions of exactly the same concentrations.

ADDITIONAL PROPERTIES OF THE THROMBOPLASTIC PHOSPHATID.

As prepared from the dried brain the thromboplastic phosphatid, after removal of fats, cholesterin, and lecithin, dissolves with some readiness when rubbed up with water, giving an opalescent solution free from visible or filtrable suspensions. From such a solution the material may be precipitated by the addition of small quantities of the neutral salts or the salts of the alkaline earths. For this reason it is advisable in making these thromboplastic solutions to use water instead of a 0.9 per cent solution of sodium chloride, since in the latter only a fine suspension may be obtained.

The aqueous solution of the phosphatid may be boiled without injuring its thromboplastic activity, but, on the contrary, it gradually deteriorates on standing. In one case, for example, an active solution of this kind was tested from time to time upon solutions of peptone plasma. When freshly made, this solution, added in equal volume, caused the peptone plasma (diluted once with water) to clot in ten to fifteen minutes. For three or four days this action was maintained with

but little visible variation, but at the end of eleven days its action had nearly disappeared. Tested upon specimens of the same plasma, there was no sign of coagulation after five hours and only an imperfect clot after twenty-four hours. The solution itself was unchanged in appearance. This result shows that a slow change of some kind gradually alters the phosphatid so as to destroy its thromboplastic power. It may also be used to refute the theory which has been proposed from time to time, especially by Nolf, that the thromboplastic action of such solutions is a physical reaction dependent upon the condition of fine suspension. In this case the physical characteristics of the solution had undergone no visible change. Indeed in many ways throughout these experiments it has been shown that the turbidity or state of suspension or opacity of the phosphatid solutions has nothing to do with their thromboplastic action. Similar solutions of egg lecithin, fine fat emulsions, etc., are entirely without action of this kind. The thromboplastic action of the tissue phosphatid (kephalin) is of a chemical rather than a physical nature.

SUMMARY.

1. A method is described for preparing a stock material of dried peptone plasma to be used in testing the thromboplastic activity of tissue extracts.

2. Dilution with water diminishes markedly the effect of the antithrombin contained in peptone plasmas, while dilution with saline solutions (0.9 per cent sodium chloride) has no such effect.

3. Ethereal solutions of dried brain or thymus gland contain a phosphatid, corresponding in its properties to kephalin, which has marked thromboplastic activity.

4. Lecithin obtained from the egg yolk has no thromboplastic action, but the yolk contains a small amount of a phosphatid, corresponding to kephalin, which exhibits thromboplastic power.

5. In saline or aqueous extracts of the dried tissues (thymus, brain) the active phosphatid is combined with a protein having a low temperature of heat coagulation, 60° C. Heating such solutions or adding acid (acetic) precipitates the protein together with the active phosphatid. The phosphatid may be extracted from these precipitates with ether, and in aqueous solution is not affected by boiling. Aqueous

Nature and Action of Thromboplastic Substance of Tissues. 21

or saline extracts of the tissues are apparently thermolabile because the active substance is carried down with the protein coagulum.

6. The phosphatid contained in the ethereal extracts of the tissues exerts its thromboplastic effect upon blood plasmas by neutralizing the action of the contained antithrombin.

7. Aqueous solutions of the active phosphatid are precipitated by the addition of neutral salts, or salts of the alkaline earths. The thromboplastic activity of the aqueous solutions disappears slowly on standing.

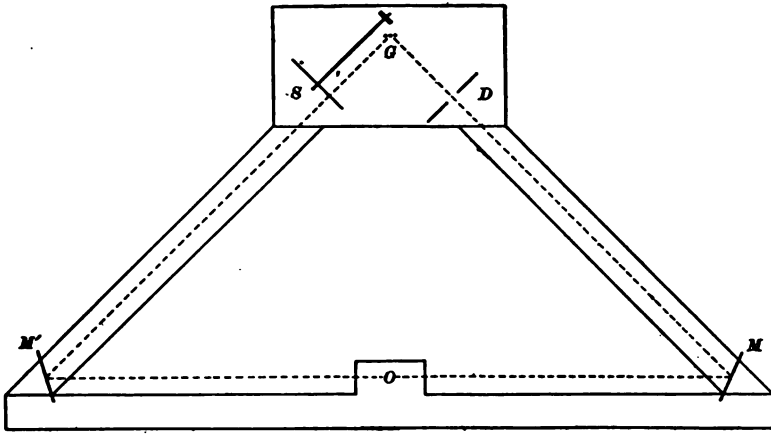
THE PHYSIOLOGICAL EFFECT OF INTERMITTENT AND
OF CONTINUOUS LIGHTS OF EQUAL INTENSITIES.

By G. H. PARKER AND B. M. PATTEN.

IN the course of the construction of a piece of light apparatus for biological experiments, it became necessary to employ some convenient means of varying the intensity of the light, and a number of devices were tried. The introduction into the beam of light of a sheet of thin glass so mounted that it could be rotated and the proportion of the reflected to the transmitted beam thus varied, failed, under the conditions of the apparatus, to give a sufficient range. A diaphragm slit of variable width, even when placed in the most advantageous position, disturbed the form of the resulting field of light too much to admit of its application. The introduction of a grating so mounted that it could be rotated in the beam of light proved to be a more suitable mechanism than the others, but it occupied an inconveniently large amount of space. None of these devices were as satisfactory, so far as compactness and ease of manipulation were concerned, as a rotating sector-wheel, which, under the name of the episcotister, has been used in many laboratories (Nagel, 1909, p. 16).

A consideration of these various means, however, will show that the light obtained by them may be physically quite dissimilar. The use of a partial reflector, like a sheet of glass, of a diaphragm slit, or of a grating to reduce the strength of the beam of light, results in the production of a continuous flow of light of low intensity, the form of stimulus desired. The revolving sector-wheel, however, does not yield continuous light, but a succession of flashes of relatively high intensity separated by short intervals of darkness, the combination of which may produce on the eye the effect of a steady flow of light of low

intensity. Since, however, this light is only *apparently* similar to that produced by the other methods and in reality possesses the striking physical peculiarity already alluded to, it is only reasonable to inquire whether or not this peculiarity has any physiological significance, before adopting the episotister as a means of reducing light. The question, therefore, that we set for ourselves was this, Is there any



observable physiological difference between the action of continuous light and of intermittent light of equal intensity? So far as we are aware, this question has never before been raised in relation to organisms.

In order to get an answer to the question just stated, we constructed a piece of apparatus, the plan of which is shown in the accompanying figure. This apparatus consisted of a horizontal wooden frame in the form of an isosceles right-triangle. The frame was so proportioned that, as shown by the dotted lines on the plan, there could be laid out on it a triangle whose longer side was 2 m. and whose shorter sides were each approximately 1.4 m. Where the two sides of the frame made a right angle with each other, a small horizontal platform, 65 cm. by 35 cm., was attached and on this was placed as a source of light three 200-volt Nernst glowers (*G*) mounted vertically and about 5 mm. apart. A portion of the light from these glowers passed through a narrow horizontal slit approximately 3 mm. wide, in the reducing diaphragm *D*, 25 cm. from the glowers, and fell upon the vertical mirror

M near the end of the arm. This mirror, which was of the best French glass, was placed at such an angle that it reflected the central ray of the beam onto the observation point, *O*, directly opposite the glowers. Another part of the light from the three glowers passed through the region occupied by the sector-wheel *S*, which was in symmetrical position in relation to the diaphragm slit in *D*, and fell upon the mirror *M'* at such an angle that its central ray was thrown upon the same observation point, *O*, as was that of the other beam, but from the opposite direction. The apparatus was so constructed that the courses taken by the light over the two paths from *G* to *O* were equal in length and symmetrical to each other. Furthermore, the two central rays impinging at *O* were in the same straight line, but, of course, opposite in direction. Light shields, not indicated in the figure, were placed around the glowers, the observation point, and on other parts of the apparatus so as to cut down reflection to a minimum, exclude extraneous light, and protect the glowers from air currents. The sector-wheel was driven by an electric motor, and could be run at rates varying from 540 to 2950 revolutions per minute. In most of our tests the rate was 1750 revolutions per minute. The sector-wheel had four radial apertures in it, situated equidistant from one another. Three of these measured 11.5° each and the fourth could be varied in size from 0° to 30° . When the sector-wheel was running, the two beams of light, opposed at *O*, were each reduced, but by different methods. The beam that passed through the slit at *D* was reduced by the narrowness of this slit and hence was a continuous flow of light of low intensity. The beam that passed through the sector-wheel was also reduced, but in the sense that it was made up of a succession of flashes and dark periods which fused indistinguishably in the eye. By varying the size of the adjustable opening in the sector-wheel, this beam could be made to agree in apparent intensity with the opposing beam from the slit, and under these circumstances the two methods of reduction could be closely studied and compared. It will be observed from the plan of the apparatus that any fluctuation in the source of the light would affect both beams equally, so that in comparing the two lights such fluctuation became negligible.

In a preliminary test a Lummer-Brodhun photometer was set up at the observation point *O*, and, by varying the adjustable aperture in the sector-wheel *S*, the light which reached the photometer through

the wheel was made equal to that which reached it through the reducing diaphragm *D*. In taking readings with the photometer, it was found that a variation of a quarter of a degree in the opening of the adjustable aperture was about the limit of recognizable difference; a change of half a degree, or approximately 1 per cent, was distinguishable with certainty. To the eye, equality was established between the two lights when the adjustable aperture in the wheel stood at 16.3° , *i. e.*, when the four apertures in the wheel together equalled 50.8° . Having established this equality, which may be called physiological equality, the reducing diaphragm and sector-wheel were now interchanged so that the beam that fell on mirror *M* was reduced by the wheel and not by the slit, and *vice versa*. A comparison of the two beams by means of the photometer still showed equality when the aperture in the wheel was 16.3° . Next the two mirrors were interchanged, but, this also did not disturb the equality. From these preliminary tests we concluded that the apparatus was balanced optically and that a difference of 1 per cent between the two lights could be recognized with certainty.

The running of the sector-wheel produced a considerable current of air, and it was suspected that this current might have a cooling effect on the glowers, thus reducing their efficiency as light producers. Although we could not find that these currents impinged in any marked degree upon the glowers, we nevertheless determined to ascertain whether this was a possible factor in influencing the intensity of the light. To accomplish this, the arm carrying the mirror *M* and the long beam on which the observation point *O* was marked were removed, and the apparatus was so placed that the beam from *M'* was directed into a radiomicrometer, the receiving disk of which occupied a position corresponding to the observation point *O*. The beam reflected at *M'* was reduced by putting the diaphragm *D* in its course, and the sector-wheel *S* was put in the path of the beam that would have gone to *M*. The intensity of the beam from *M'* was now measured, first, with the wheel running, and, secondly, with it still. The intensity of the beam with the wheel running was found to be 9.10 scale units, and with the wheel still 9.08. Several subsequent tests gave similar results, and we therefore concluded that, notwithstanding the fact that the running of the wheel produced an air current, this current did not disturb appreciably the amount of light generated by the glowers.

Having tested the apparatus in the manner described, we now proceeded to a comparison of the two kinds of reduced light. The lights were balanced physiologically by setting the adjustable aperture in the sector-wheel at 16.3° , the position which, as already noted, gave equality of illumination when tested with the photometer, and we now proceeded to measure the intensity of the two lights by means of the radiomicrometer. One arm and the long beam of the apparatus were removed, and the mechanism thus reduced was so placed that the light from mirror M' was thrown upon the radiomicrometer disk, which occupied a position corresponding to the observation point O . This light was then reduced by placing in its path, 25 cm. from the source, the diaphragm D with its slit of 3 mm. width, and the intensity was measured by the radiomicrometer. Next the sector-wheel with its adjustable aperture at 16.3° was substituted for the diaphragm, and the light thus reduced was again measured by the radiomicrometer. In this way there was obtained an intensity determination for each of the two lights which to the human eye appeared equal. The determinations were based upon readings that were made in a basement room of the laboratory at night, usually about midnight, when mechanical vibrations were mostly absent and when the radiomicrometer showed the least drift. A damp atmosphere with no wind seemed to be the most favorable condition for uniform readings. Care was taken that the illuminating current remained constant at 200 volts, and the readings were made between groups of readings of a standard incandescent lamp on a 100-volt current. In all, five sets of determinations were made. These are given in Table I.

The following table shows that in all pairs of reduced lights judged as equal in intensity by means of the photometer, that member of the pair which was reduced by the sector-wheel was always of higher intensity than the other. The difference of the averages of the sets of pairs is 0.52 scale units, or about 5.9 per cent. This difference we believe to be a true difference in the two fields of light and not due, for instance, to a heaping up of the energy of the light in one field as compared with the other, as by diffraction, for, though our radiometric measurements in the regular tests were made at one spot close to the centre of each field, a radiometric exploration of the fields showed a central area many centimetres square and uniform in light intensity. As the average probable error of the series of readings in the table is

less than 1 per cent, we are justified in concluding from these observations that a continuous flow of light is a more efficient stimulus than an intermittent flow, even though the latter delivers to the receptive surface somewhat more energy per unit of time than the former.

At this step it is natural to raise the question whether the rate of rotation of the sector-wheel has any appreciable influence on the

TABLE I.

AVERAGE MEASUREMENTS, IN ARBITRARY UNITS OF A SCALE, OF THE INTENSITY OF A BEAM OF LIGHT REDUCED TO PHOTOMETRIC EQUALITY FOR THE HUMAN EYE (1) BY A SECTOR-WHEEL (FLASHES OF INTENSE LIGHT ALTERNATING WITH DARKNESS) AND (2) BY A NARROW SLIT (CONTINUOUS FLOW OF LIGHT OF LOW INTENSITY).

Numbers of pairs of measurements.	Light reduced by		Differences.	
	Sector-wheel.	Narrow slit.	Actual.	In per cent.
1	9.40 \pm 0.061	8.87 \pm 0.048	0.53	6.0
2	9.40 \pm 0.027	8.93 \pm 0.030	0.47	5.3
3	9.56 \pm 0.068	8.96 \pm 0.014	0.60	6.7
4	9.21 \pm 0.053	8.71 \pm 0.018	0.50	5.7
5	9.41 \pm 0.048	8.91 \pm 0.079	0.50	5.6
Averages.	9.396	8.876	0.52	5.9

apparent intensity of the intermittent light. To answer this query we set the sector-wheel at physiological equality and ran it at 775, 940, 1200, 1750, and 2950 revolutions per minute. At these several rates we were unable to detect in the photometer any disturbance of physiological equality. Readings in the radiomicrometer at 540, 1750, and 2950 revolutions per minute were also not significantly different. We therefore concluded that for our investigations the rate of rotation, at least between 540 and 2950 per minute, was not a significant factor; in continuing our observations, we therefore used 1750 rotations per minute as the usual rate.

If the light reaching the radiomicrometer through the combined openings in the sector-wheel of 50.8° is 5.9 per cent too strong for physical equality with that which reached the radiomicrometer through the

slit, to establish physical equality it would be only necessary to reduce the aperture in the wheel by 5.9 per cent, *i. e.*, this aperture should be changed from 16.3° to 13.5° . On comparing the intensities of the light as reduced by the slit and by the sector-wheel with its adjustable aperture at 13.5° , it was found that the light through the slit had an intensity, as shown by the radiomicrometer, of 8.95 scale units and that through the wheel of 8.97 units, *i. e.*, the two lights exhibited what was essentially physical equality. On recombining the apparatus and placing the photometer at the observation point, the beam of light that emerged through the slit was easily seen to be a brighter light than that which emerged through the wheel, showing that of two lights of equal physical intensity that which is delivered as a continuous flow is more efficient as a stimulus than that which comes to the eye as rapid flashes.

From the two sets of observations recorded in the preceding paragraphs, we believe that we are justified in concluding that, other things being equal, an intermittent light is measurably less efficient as a stimulus for the eye than a continuous light. So far as we have been able to ascertain, this determination has never before been made for the eye, and its only recorded parallel is to be found in certain statements made concerning photochemical surfaces. The so-called photographic or Bunsen-Roscoe law states that for a given photochemical material the time of exposure multiplied by the intensity of the light yields a constant quantity. It is well known that this so-called law is only approximately accurate. That the law applies to organisms much as it does to photographic surfaces has been pointed out by Fröschel (1908) and Blaauw (1909), as well as by Loeb (1911, p. 465). As applied to photographic papers, etc., the Bunsen-Roscoe law has been shown to be open to exceptions by Abney (1890, p. 481; 1907, p. 391) and by Englisch (1899, p. 117; 1900, p. 131), both of whom have, apparently independently, demonstrated that an intermittent light has less effect on a photochemical substance than a continuous one of equal intensity. This condition is exactly parallel with what we have found for the human eye, and suggests at once that the human retina and probably the light receptors of other organisms are organs that are based in their action upon chemical principles not unlike those of the photographic plate.

Why it is that both the eye and photochemical materials receive less

effect from an intermittent light than from a continuous one of equal intensity is still a matter of uncertainty, but it is not improbable that in both cases the chemical changes initiated by the light acquire their full velocity only slowly, and that when this velocity is repeatedly interrupted and revived, as in the case of intermittent light, the change on the receptive surface is less for the amount of energy supplied than when, as in the case of continuous light, the change is once for all initiated and then allowed to proceed without break. Expressed in another way, we may say that both photochemical materials and the eye exhibit an amount of chemical induction that becomes measurably observable in the greater effect produced on these receptive surfaces by continuous light than by intermittent light of equal intensity. We also conclude that the episcotister is an unreliable means for the accurate reduction of the intensity of light.

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THE COURSE OF THE WAVE OF NEGATIVITY WHICH PASSES OVER THE TORTOISE'S HEART DURING THE NORMAL BEAT.

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OUR problem has been to find the origin and trace the course of the negative variation which sweeps over the heart at the beginning of each cardiac cycle. This condition of negativity, passing successively from point to point, has been interpreted by most electrocardiographic workers as due to the activity of the contracting muscle. The question once before debated in regard to skeletal muscle has, however, been raised in the case of the heart, namely, whether this initial wave of negativity may not be due to conduction rather than contraction. Experiments of Judin,¹ Hoffmann,² De Meyer,³ and Trendelenburg,⁴ as well as some investigations of our own, have rather strongly supported this view. For the present, however, we need not commit ourselves in regard to the exact cause of the wave of negativity which we have studied. If it should be shown to be produced by processes of conduction, it is at least followed after a very brief latent period by contraction. Negativity of a given area on the surface of the heart is an indication that under normal conditions that part will soon enter into contraction. This statement is particularly true of those hearts like the cold-blooded in which the musculature has not been arranged in various strata and in which no specialized conduction system has developed. While, strictly speaking, we have traced only the course of a wave of negative variation over the heart, we have in effect followed the course of the wave of contraction.

¹ JUDIN: *Zentralblatt für Physiologie*, 1908, xxii, p. 365.

² HOFFMANN: *Archiv für die gesammte Physiologie*, 1910, cxxxiii, p. 552.

³ DE MEYER: *Archives internationales de physiologie*, 1907, v, p. 76.

⁴ TRENDLENBURG: *Archiv für die gesammte Physiologie*, 1912, cxliv, p. 39.

The principle that initial negativity means initial activity has been used by many investigators to determine which part first entered into action. Waller⁵ and Bayliss and Starling⁶ sought by this method to discover whether contraction began in base or apex of the mammalian ventricle. Wybau⁷ and Lewis⁸ in the same way determined the site of the pacemaker in the mammalian heart. Gotch⁹ used essentially the same method in studying the succession of events in the contracting ventricle.

Our method, then, has been to find the order of negativity in the different parts of each segment of the heart. To accomplish this we have led off from the superficies of the heart to the string galvanometer. Tortoises of the genus *Chrysemis*, 6 to 12 inches in diameter, were used. The plastron was removed and the heart exposed by opening the pericardium. Non-polarizable zinc electrodes were used which consisted of glass tubing plugged with a saline mash of filter paper and filled with zinc sulphate. From the mash of filter paper a woollen thread was hung, the tip of which rested on the heart at the point desired. This insured a good localized contact, the extent of which was unaffected by the movements of the heart. The electrodes were connected in the usual way with the large model Einthoven string galvanometer. Records were made by means of the photographic recording apparatus on bromide paper.

The electrodes were labelled right and left hand, negativity of the former resulting in an upward movement of the string as its shadow was recorded by the photographic apparatus, and negativity of the latter resulting in a downward movement. The time was recorded in one-fifth second intervals at the top of each record. The instant of contraction of the heart or the chamber under observation was signalled by the observer pressing a bulb which moved a tambour lever in front of the photographic slit. This signal served merely to identify the electrical wave which was produced by the particular chamber

⁵ WALLER: Philosophical transactions of the Royal Society, B, 1889, clxxx, p. 169.

⁶ BAYLISS and STARLING: Internationale Monatsschrift für Anatomie und Physiologie, 1892, ix, p. 256.

⁷ WYBAU: Archives internationales de physiologie, 1910, x, p. 78.

⁸ LEWIS, OPPENHEIMER and OPPENHEIMER: Heart, 1910, ii, p. 147.

⁹ GOTCH: Heart, 1909, i, p. 235.

under observation. This method avoided the harmful manipulation which would have been necessary in connecting the heart to a receiving tambour.

As far as possible the leads were taken without disturbing the heart or moving it in any way. To reach the posterior surface, it was of course necessary to raise the heart by means of a ligature attached to the frænum. Contacts around the auriculo-ventricular ring also necessitated cutting the aortæ and pulling the auricles out of the way. These manipulations were made with care, and as little injury as possible done to the heart. Our experience has been that handling the heart leads to irregularities. The exceptions in our results can undoubtedly in large measure be attributed to this factor. All leads from the anterior surfaces were taken first, and only after these was the heart raised to expose the posterior surface and the sinus region. The order of discussion in the text does not therefore indicate the exact order in which the observations were made.

In regard to the interpretation of the curves, the first clear cut, quick movement above or below the abscissa was taken to indicate the potential of the tissues under the electrodes. Usually this movement occurred without any small preliminary oscillations. In certain cases, however, such small preliminary waves did occur either in the same or in opposite direction to the main movement. These irregularities have, of course, been disregarded in our interpretation of the curves, since their origin was obvious. If a distant point became negative before either of the areas under the electrodes, it was possible that the electrode nearest the point of negativity might show a slight change in potential, since the tissue of the heart acts as an indifferent conductor. These primary deviations occurred infrequently and for the reasons given have been disregarded.

SINUS AND RIGHT VENA CAVA.

In the days of Haller, as is well known, the origin of the beat in the cold-blooded heart was believed to be in the venæ cavæ. From here it spread in regular order over the heart. From the time of Schiff, however, physiologists have in most cases assigned the origin of the beat to the sinus venosus. McWilliam,¹⁰ referring to the heart of the

¹⁰ McWILLIAM: *Journal of physiology*, 1885, vi, p. 199.

eel, said that in the intact heart the normal systole began by a distinct simultaneous beat in the right and left ostial parts of the sinus. Engelmann¹¹ believed that in the intact frog's heart the veins with the sinus acted as a unit and beat synchronously. Gaskell¹² always declared that the sinus was the part of the heart from which the rhythmic beat took its origin. Recently Garrey¹³ has published an article on rhythmicity in the turtle's heart, in which he has come to

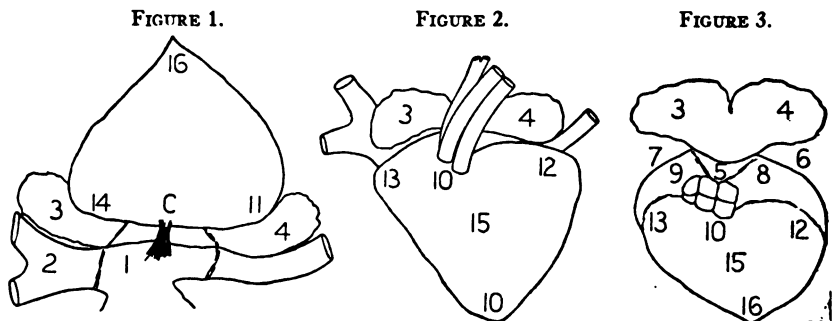


FIGURE 1. — The heart with apex raised in order to show the sinus (1), right caval vein (2), and posterior surface of the ventricle.

FIGURE 2. — Anterior face of the heart.

FIGURE 3. — Anterior view of the heart with the aortæ cut and the heart pulled forward to expose the auriculo-ventricular ring and the basal portions above the lateral margins of the ventricle. The numbers refer to the position of the electrodes, and their numerical order is that in which the parts became negative.

the conclusion that the right vein precedes the sinus in point of contraction. His conclusions were based on inspection and on curves taken by the suspension method from the right and left caval veins.

In our work we have compared the potential of the right caval vein and sinus at the beginning of the cardiac cycle. The ventricle was raised to expose the parts beneath. In Fig. 1 is shown schematically the appearance of this region. The location of the electrodes is marked in this diagram by the numbers 1 and 2. The right-hand electrode was placed on the sinus so that initial negativity of that region would be indicated by an upward stroke in the record. In all fifteen records were taken. In twelve of these the string responded with an upward movement, indicating primary activity in the sinus. In two experiments the results were doubtful, and in the

¹¹ ENGELMANN: *Archiv für gesammte Physiologie*, 1896, lxx, p. 115.

¹² GASKELL: *Journal of physiology*, 1883, iv, p. 43.

¹³ GARREY: *this Journal*, 1911, xxviii, p. 330.

remaining one the vein clearly preceded. These results seem to show pretty conclusively that according to the string galvanometer negativity appears first in the sinus. One of our records is reproduced in Fig. 4. To secure this curve the string was made more than usually sensitive.

It will be noted that in our experiments the sinus electrode was placed somewhat to the right of the mid line. The results therefore indicate that the point of primary negativity is in the region of the right cavo-sinal junction, a finding which is in harmony with McWilliam's experiments and analogous to all the recent work showing that in the mammalian heart the pacemaker resides in the right side at the junction of vein and auricle, that is, in the so-called sinus region.

Our results are at variance with Garrey's¹⁴ work on the turtle. This author found that the beat originated in the right veins, and that there was a distinct pause before the contraction reached the sinus. These conclusions were based on inspection and on curves taken from the right caval veins by suspension methods. The manipulations necessary to secure records by suspension may have had something to do with causing Garrey's results to differ from our own, for we have on several occasions noted that the time relation of two regions might be reversed by slight injury to the tissues under one of the electrodes. It seems, however, that the principal explanation of the differences between Garrey's work and our own may well be due to the methods employed. The origin of the beat in any part of the heart must not be confused with movement of that part, yet it may be impossible to avoid this error when using methods of inspection and suspension. The physical configuration of a part may prevent its exerting traction on a lever at the moment it enters into activity. Or, what is more important, the beat may actually arise in a part that gives no sign of contraction to the eye whatever. In very few tortoise hearts is it possible to identify the beat of the sinus. To all appearances the beat does start in the veins, and yet when the electrodes of the galvanometer are applied to the parts evidence of primary activity is found in the very tissue that seems sometimes quiescent to the eye. It was mainly for these reasons that we have used the string galvanometer in a problem of this kind. It seems to meet every requirement of investigations of this nature.

¹⁴ GARREY: *Loc. cit.*

RIGHT AND LEFT AURICLES.

By means of tissues in the sino-auricular junction the excitation wave reaches the auricles. To all appearances the auricles contract simultaneously, but so far as we are aware no one has examined this point carefully in the cold-blooded heart. Fredericq¹⁵ and his school have presented evidence showing that the right auricle of the dog precedes the left by an interval of two to three hundredths of a second. These results are to be expected *a priori* if the wave of contraction starts from the sinus region and spreads over the heart, unless indeed some mechanism has been developed to hold in check the parts nearest the pacemaker.

In our experiments leads from the auricles were made as indicated in Fig. 2, the right-hand electrode being on the left auricle (4) and the left-hand electrode on the right auricle (3). In all twenty-four observations were made. In twenty of these the right auricle preceded the left, in two the left preceded the right, and in two the curves could not be interpreted with certainty. Our results therefore seem to be in agreement with what has been found for the mammalian heart.

Fig. 5 presents a curve taken from the two auricles. The left-hand electrode being on the right auricle, the sharp down stroke of the curve indicated primary negativity in that chamber. That mutilation of the heart may disturb the regular sequence of events in the beating heart has been shown several times in our work with the auricles. In one case we have records showing that in the uninjured heart the right auricle contracted first, but after cutting away the ventricle the left auricle began to lead. Examples of this kind emphasize the care that must be exercised in this kind of work and also explain some of the exceptions noted. Since some injury to the heart is always necessary and unavoidable, we have tried to make up for the fact by making a large number of observations, in the belief that a majority of the results would indicate the true state of affairs.

COURSE OF THE WAVE OF NEGATIVITY OVER THE VENTRICLE.

To determine the sequence of events in the ventricle we have made use of a large number of leads which are indicated in the three dia-

¹⁵ FREDERICQ: Archives internationales de physiologie, 1906, iv, p. 57; SCHMIDT-NIELSEN: Archives internationales de physiologie, 1907, iv, p. 417.

grams of the heart (Figs. 1, 2, and 3). The principal points on which electrodes were laid were the anterior (5), left (6), and right (7) quarters of the auriculo-ventricular ring, the right (13) and left (12) anterior, and the right (14) and left (11) posterior ventricular bases, the aortic base (10), points on the ventricular base above the lateral margins (8 and 9), the middle of the anterior surface of the ventricle (15), and the apex (16). By placing the electrodes on any two of these

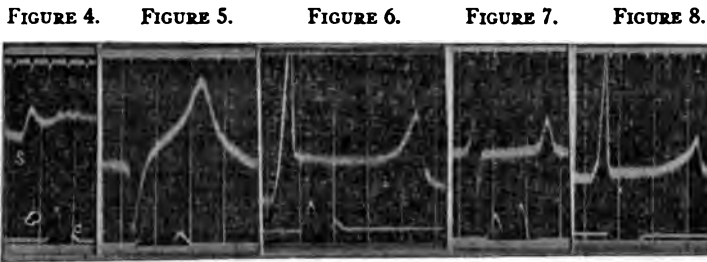


FIGURE 4. — Record showing origin of beat in sinus. Electrodes on sinus (1) and right caval vein (2). Up stroke indicates initial negativity of right-hand electrode which was on sinus. Signal at bottom, in this and all following records, shows the time of contraction of the chamber under study, as noted by the observer.

FIGURE 5. — Record from the two auricles. Left-hand electrode on right auricle; hence sharp down stroke indicates primary negativity of that chamber.

FIGURE 6. — Curve showing negativity of aortic base to apex. Up stroke indicates negativity of right-hand electrode which was on aortic base.

FIGURE 7. — Curve showing aortic base negative to right anterior base. Up stroke indicates negativity of right-hand electrode which was on aortic base.

FIGURE 8. — Record showing right posterior base negative to apex. Up stroke indicates negativity of right-hand electrode which was on right base. These records are one half the original size.

regions we could of course determine which point showed negativity first. Instead of taking each point separately and comparing it with all the others we found it more expeditious to determine first the order of negativity in each of the three important regions, that is, the anterior surface, the posterior surface, and the auriculo-ventricular ring. This being done, it was easier to compare the points of primary activity with each other and thus determine the sequence for the entire ventricle.

The anterior surface of the ventricle was first studied. Three different regions of the ventricular base were compared with the apex. These regions, shown in Fig. 2, were the right ventricular, the aortic, and the left ventricular bases. In all, seventeen observations were

made from right ventricular base and apex, twenty-one observations from aortic base and apex, and twenty-eight from the left ventricular base and apex. Fig. 6 illustrates negativity of aortic base to ventricular apex. Of these sixty-six records every one without exception has shown the base of the tortoise's ventricle to be negative before the apex, irrespective of what basal part was chosen for the comparison.

The sequence in the basal parts of the anterior surface was next investigated. Leads from the right and left bases showed that out of twenty-six observations the left side preceded the right in negativity nineteen times, the right preceding the left only seven times. One doubtful record has been credited to the right base. From this data it would seem that the left anterior base normally precedes the right. The aortic base was next compared with the left base. In twenty-one trials with only one exception the aortic base preceded the left. The accuracy of these determinations was in a measure checked by comparing the aortic base with the right base. Since the left base preceded the right, and the aortic base preceded the left, it was of course to be expected that the aortic would also precede the right base. This was found to be true twenty out of twenty-one times. Fig. 7 reproduces a curve illustrating this point. From the data thus far presented the order of negativity on the anterior surface of the ventricle is known to be as follows: aortic base, left anterior base, right anterior base, and apex.

The points studied on the posterior surface of the ventricle are shown in Fig. 1. They included the right and left bases (14 and 11), the middle of the base (*c*) and the apex (16). The right and left bases were first compared. Out of twenty-seven observations twenty-two showed the left base negative before the right. The right base, however, preceded the apex in each of six records. Fig. 8 shows the initial negativity of the left posterior base to the opposite posterior side. The middle of the posterior base (*c*) was found to follow the left base in order of negativity. This observation was of interest for the point (*c*) was in close proximity to the posterior portion of the auriculo-ventricular ring.

It was now possible to compare the anterior and posterior surfaces as a whole. Observations thus far had shown that activity first appeared on the anterior surface at the aortic base and on the posterior surface at the left base. Records were accordingly taken from these

two points. Out of eighteen observations sixteen showed that the aortic base became active before the left posterior. Fig. 9 is a reproduction of one of these records. The left posterior base was then compared with the second point of negativity on the anterior surface, the left anterior base, and found to precede the latter fifteen out of twenty-three times. The right posterior and anterior bases were then compared. In fourteen out of eighteen observations the right anterior base was found to be negative before the right posterior. The sequence of negativity over the entire body of the ventricle could now be stated. The aortic base preceded all points on the anterior surface as well as the primary point of the posterior surface, the left base. The left posterior base in turn preceded all other parts of the posterior base and the left anterior base. The left anterior base preceded the right anterior base, and the latter in turn preceded the right posterior base. All parts of the ventricular base preceded the apex.

The order of negativity thus far determined for the ventricle was, therefore, aortic base, left posterior base, left anterior base, right anterior base, right posterior base, and apex. This order was verified by numerous checks. The left posterior base was compared with the right anterior base and found to precede it nine out of ten times. The left anterior base preceded the right posterior base seventeen out of twenty-two times. The aortic base was found negative to the right posterior base without exception.

The auriculo-ventricular ring now remained to be examined and brought into relation with the parts of the ventricle below it. In order to apply the electrodes the aortæ were transected and the heart pulled slightly upon its apex. The anterior and lateral portions of the ring were thus exposed as indicated in Fig. 3. The posterior part of the ring was not considered at this time, for, as has been stated already, negativity in that region (*c* of Fig. 1) did not appear until after the left posterior base. Leads were first made from the right (7) and left (6) sides of the auriculo-ventricular ring. In all twenty-two observations were made, and of these seventeen showed primary activity in the left ring. The anterior portion of the ring was next compared with the left side. The observations numbered sixteen and without exception they showed that the anterior half of the ring was first negative. Fig. 10 presents one of these records. The aortic base, the first point to become negative in the body of the ventricle, was

now compared with the right half of the ring which was the last point of the ring to become negative. Out of seventeen records taken fourteen indicated initial negativity in the right half of the ring. The accuracy of these results was tested by control leads wherever possible. For example, the anterior portion of the auriculo-ventricular ring preceded the aortic base without exception.

The data presented in the preceding description of our experiments has enabled us to state the order in which negativity appears in the ventricular regions studied. It is as follows: anterior half of the auriculo-ventricular ring, left half of the auriculo-ventricular ring, right half of the auriculo-ventricular ring, aortic base, left posterior base, left anterior base, right anterior base, right posterior base, apex.

DISCUSSION.

Our work seems to confirm the older workers who believed that contraction in the tortoise heart was a wave that swept from sinus through auricles and ventricle, terminating finally at the apex. This was Gaskell's well-known conception of the events of the cardiac cycle. Our experiments have agreed with Gaskell's in a number of interesting details, particularly in regard to the primary point of negativity in the ventricle being in the anterior half of the auriculo-ventricular ring. Gaskell tested out the importance of the four quarters of the auriculo-ventricular ring and found that the rhythm was most disturbed when the anterior quarter was severed. Very little or none of the functionally connecting tissue between auricle and ventricle seemed to lie in the posterior portion of the ring and but little more in the lateral portions. When the anterior part, on the contrary, was mutilated, the contraction had difficulty in passing from auricle to ventricle. In agreement with these observations is our result showing that the first point of the ventricle to become negative at the beginning of the ventricular systole is the musculature immediately adjacent to the anterior part of the auriculo-ventricular ring.

Our conception of the course of the wave of negativity and contraction over the ventricle is that it spreads downward from the circular fibres of the auriculo-ventricular ring over the entire basal musculature of the ventricle. The fact that the aortic base becomes negative before the left posterior base and the latter becomes negative before the right posterior base does not mean that the wave passes

first to the aortic base, and then to the left base and later through the heart to the right. It means rather that as the wave sweeps downwards over the ventricle it happens to reach the aortic base first and then the left base before the right. In support of this we have found that points between the left base and the ring are negative to all points below base. Leads from the left base (12) and a point above the margin of the anterior surface (8) show that the region nearer the ring is negative first. This point is also negative to the aortic base.

FIGURE 9. FIGURE 10. FIGURE 11. FIGURE 12. FIGURE 13.



FIGURE 9. — Curve showing aortic base negative to left posterior base. Right-hand electrode on aortic base. T wave is not included.

FIGURE 10. — Record showing anterior portion of the ring negative to left side. Left-hand electrode on anterior part of ring.

FIGURE 11. — Record showing curve taken with right-hand electrode across the entire ventricular base and left-hand electrode on apex.

FIGURE 12. — Record showing curve taken with base-apex leads after the entire aortic base had been cut away. Presence of the final variation is to be especially noted.

FIGURE 13. — Record taken with right-hand electrode across the entire base and left-hand electrode on middle of anterior surface of ventricle. These records are one half the original size.

It seems then that negativity in the basal portions is largely determined by proximity to the ring. This does not exclude certain pathways by which the wave may pass more directly or with greater ease, since the fact that the left side of the heart precedes the right is evidence of just such paths, but it does seem to mean, on the whole, that the course of the wave is a downward one from the auriculo-ventricular ring over the basal musculature of the heart.

The most important question in connection with the sequence of events during ventricular contraction is whether there is a return of the contraction wave to the base. All workers are now agreed on the general form of the ventricular part of the electro-cardiogram, which consists of two positive waves, the R and T according to the nomenclature of Einthoven. Many ideas indeed have been advanced con-

cerning the origin of the final variation or T wave. Two of these have received a great deal of attention. The first was proposed by Bayliss and Starling.¹⁶ These authors found that in the intact dog and man the electrical variation was triphasic, first an up stroke indicating base negativity, second a downward movement indicating activity in the apex, and third a final upward stroke showing that negativity was again dominant at the base. This final variation, now known as the T wave, they believed to be due to the fact that the excitatory state at the base outlasted that at the apex. As is well known, this general idea has in later years been made use of by Einthoven in his explanation of the T wave of the electro-cardiogram.

The second interpretation of the T wave of which we wish to speak is that outlined by Gotch¹⁷ first in 1907, and elaborated in detail in 1909. Gotch found for the first time that the cold-blooded heart *in situ* gave the same curves as the heart of mammals. By studying curves taken with the electrodes on the superficies of the tortoise's and rabbit's heart Gotch developed the following idea of the sequence of events during the ventricular systole. The contraction wave originated in the basal portions of the ventricle, particularly the venous regions, thence it was propagated to the apex, and then by cross routes it returned to the aortic exit at the base, this part being the last to develop activity. This theory agreed with the ideas already expressed by Nicolai, and it has since been made much of by Kraus and Nicolai in their interpretation of the electro-cardiogram.

So far as the cold-blooded heart is concerned Gotch's ideas rest on the interpretation of curves taken from three different leads. Curves with the electrodes on the external border, near either the right or left anterior bases, and apex were simple diphasic curves which presumably meant that the contraction wave had passed from base to apex. When the basal electrode, however, was placed at the aortic exit a triphasic curve resulted, which apparently meant that the base was first negative, then apex negative, then both base and apex at the same potential, then apex alone negative, and finally the base again negative. The ventricle was therefore believed to be the seat of two active states, one of which was immediate, the other delayed. The immediate effect alone was present in tissue adjoining the auric-

¹⁶ BAYLISS and STARLING: *Loc. cit.*

¹⁷ GOTCH: Proceedings of the Royal Society, *B.* 1907, lxxxix, p. 323.

ulo-ventricular ring, as shown by the diphasic curves from left anterior base and apex. The aortic base, however, was the seat of a delayed activity. The contraction wave was thus conceived to be propagated from the auriculo-ventricular ring to the apex and back again to the aortic base. If the view proposed was correct, Gotch decided *a priori* that when the electrodes were placed on the right lateral base and the aortic base, a diphasic curve should result showing activity first in the right base and later in the aortic region. Just such curves were secured, although not invariably.

As can be seen by reference to the curves which we have already presented, our results have been at variance with those of Gotch in rather important particulars. The records which we have secured from the various base-apex leads have resembled greatly those which Gotch observed by laying the one electrode across the entire ventricular base and the other on the apex. We refer to Fig. 3 of his paper. We agree that the base always shows initial activity, but we have found no evidence supporting the view that the final variation is due to a return of activity to the base.

Gotch found that a lead from the lateral bases and apex gave a diphasic curve, while a lead from the aortic base and apex always yielded a triphasic curve. Our records from these two leads have been practically identical. Fig. 6 illustrates the general type. Each one has shown an up stroke, then a partial return to the abscissa and a final up stroke. According to the interpretation offered by Gotch there would then have to be a return of the contraction wave to all parts of the base, a conclusion of course not in agreement with the theory itself.

Gotch supposedly clinched his arguments when he showed that leads from the right lateral base and aortic base of the tortoise gave a diphasic curve with initial negativity in the region of the lateral base. In this we have not been able to confirm him. Out of twenty-one observations the string galvanometer has shown the aortic base to be primarily negative twenty times, yet according to Gotch's hypothesis the left-hand electrode which was on the path from the auriculo-ventricular ring to apex should have shown negativity first. Fig. 7 may be referred to in illustrating this point.

The theory that the final variation is due to a return of activity to the base has been subjected to a number of crucial tests by us,

none of which it seems to have met in a satisfactory way. In the first place, this final variation in base-apex leads is not always positive, as it must be if it is due to a return of activity to the base. The majority of curves taken from base and apex are similar to that already shown in Fig. 6, but often from a perfectly normal heart, at least so far as can be told by other leads, we have secured a curve with a negative T. Fig. 11 illustrates a case of this kind. The right-hand electrode lay across the entire base, and a return of contraction to the base should therefore have resulted in a final upward movement. The leads were identical with those by which Gotch secured his Fig. 3, yet in these cases, while the usual result is similar to that of Gotch, we have often secured a negative T.

We have also taken curves with electrodes on the base and apex after the aortic region had been killed by heat or entirely cut away. Large areas were destroyed in these experiments, yet there was always a final variation, showing as it were a return of the contraction to a base which no longer even existed. Fig. 12 reproduces such a curve taken with leads from base and apex after the entire aortic base had been removed.

If the T wave were really due to a return of contraction from apex to base, it seemed to us that when the right-hand electrode was placed on the base and the left-hand electrode rested on the middle of the anterior surface half-way between base and apex (15 on Fig. 3), we should get a curve with a diphasic R and a negative T. Initial negativity in the base would result in an up stroke, and as the wave passed by the left-hand electrode on the middle of the auricle this would become negative and give a down stroke. When the wave returned toward the aortic base, the left-hand electrode would be first affected and we should expect a final down stroke completing the cycle. Fig. 13 is a reproduction of a curve taken with the electrodes in the positions just described. As can be easily seen, our reasoning in regard to the R wave was justified by the curve, but the final variation was positive and not negative, as required by the hypothesis under question.

The long delay which takes place before the supposed return from apex to base also seems to us to be an objection to the theory under discussion. Our records, which agree with those of Gotch in this particular, show that the contraction wave passes from base to apex in about two tenths of a second. This is at the rate of 125 mm. per

second, the ventricles being about 25 mm. long. After negativity has reached the apex as determined by the down stroke of the R wave, it is anywhere from six tenths to one second before the final variation begins. This means that there must have been a delay of four tenths to eight tenths of a second at the apex or somewhere between apex and base, yet we know of no mechanism by which any such delay could take place, especially in view of the well-known fact that strips of the tortoise's ventricle conduct with equal ease in all directions.

It does not seem possible that any considerable body of musculature such as would be necessary to produce the electromotive force of the T wave could remain uncontracted near the origin of the aorta without being greatly distended by the ventricular pressure which of course rises before that time. This distention would be visible to the eye, but such a bulging is never seen. A distention does sometimes momentarily occur in the lower lateral parts of the ventricle, but seldom if ever around the aortic exit. It might be assumed that a second sheath of muscle had entered into contraction to prevent any such distention, but such an assumption would be directly against the underlying principle of Gotch's whole theory, that is, that the contraction wave follows the general course of the embryonic tube from which the heart has developed.

Another objection to the T wave being considered as a return of the contraction wave to the base has appeared in the case of extra systoles produced during vagus inhibition. With the heart in complete inhibition extra systoles have been produced by mechanical stimulation of the aortic base. This would involve all the musculature of that region, the contraction would spread to the apex, and with a base apex lead one would expect, according to the theory, a mono- or diphasic curve without any final variation, since all the basal parts have already been in action. But in all the curves we have never failed under these conditions to secure some kind of a T wave.

A final objection to looking on the T wave as a return of contraction to the base is found in the fact that no matter on what parts of the ventricle the electrodes may be placed there is always a final variation or T wave. A return wave can thus be proved to any part, even the apex in case of extra systole from that point. We have already referred to a number of different leads in which this is true, but the matter may be carried even further. A strip of ventricular muscula-

ture, no matter from what part of the heart it may be secured, on stimulation always shows an initial variation, usually diphasic, followed by a final variation or T wave. A small piece of the apex 1 cm. long, a strip cut transversely from the body of the ventricle, shows the same phenomenon. Reference might be made here to the fact that Gotch found a T wave in the auricular part of his curves from the intact animal. That the auricle gives a T wave we can abundantly confirm.

These objections seem to us to render a theory of the T wave based on the return of the contraction wave to the aortic base of the heart entirely untenable, at least in the cold-blooded heart. We hope to develop our ideas in regard to the T wave in another connection. It may be sufficient to emphasize the fact here that a T wave is found in all curves secured by leading off directly from cardiac muscle. The data suggest that the T wave may be due to differences in intensity or duration of the processes incident to contraction under the two electrodes.

SUMMARY.

1. The course of the wave of negativity which appears at the beginning of each cardiac cycle has been traced over the tortoise's heart. The method used has been to compare the potential of different points on the superficies of the various chambers, leading off with non-polarizable electrodes to the string galvanometer and recording the movement of the string photographically. The order in which negativity appears over the heart has been shown to be the following: sinus, right vein, right auricle, left auricle, anterior part of auriculo-ventricular ring, left part of auriculo-ventricular ring, right part of auriculo-ventricular ring, aortic ventricular base, left posterior base, left anterior base, right anterior base, right posterior base, and apex.

2. Our work seems to confirm the older workers who believed that contraction in the tortoise's heart is a wave that sweeps over sinus, auricles, and ventricle, terminating at the ventricular apex.

3. Experimental evidence has been presented showing that the T wave or final variation cannot be done to a return of the contraction from apex to aortic base. The T wave is characteristic of cardiac muscle, and is probably due to differences in duration or intensity of the processes incident to the contraction under the two electrodes.

THE EFFECT OF CARBON DIOXIDE AND OF OXYGEN UPON MUSCULAR TONE IN THE BLOOD VESSELS AND ALIMENTARY CANAL.

By D. R. HOOKER.

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INTRODUCTION.

IN 1901 Bayliss¹ reported the observation that carbon dioxide causes vascular relaxation when added to Ringer's solution, perfusing the limb vessels of frogs. A year ago the present author readily substantiated the correctness of this observation,² and showed further its physiological corollary, namely, that oxygen under similar conditions produces an improvement of vascular tone. It was further stated at the time that this reaction of the vascular muscle is not confined to blood vessels *in situ*, but is exhibited as well in ring preparations of arteries and veins suspended in atmospheres of oxygen and carbon dioxide and may be shown equally well in preparations obtained from warm and cold blooded animals.

In view of the emphasis which has of late been put upon the importance of carbon dioxide to the animal body, and especially upon its importance as a chemical regulator of vascular and tissue processes,³ these observations appeared worthy of more detailed investigation.

It is evident that excessive amounts of a normal constituent of the body may react deleteriously to the tissues. Thus carbon dioxide might well, as above indicated, cause in a saturated atmosphere or solution a depressant action, while in amounts approaching the normal it might be beneficial or stimulant in action. It is, accordingly, of importance to know what differences, if any, may be observed in such tissue when

¹ BAYLISS: Journal of physiology, 1901, xxvi, p. xxxii.

² HOOKER: this Journal, 1911, xxviii, p. 361.

³ HENDERSON and UNDERHILL: this Journal, 1911, xxviii, p. 275; also earlier papers by HENDERSON.

exposed to gas mixtures in which the percentage of carbon dioxide is varied. In this connection the work of Jerusalem and Starling⁴ on the mammalian heart will be recalled. They found that carbon dioxide, 5-8 per cent in an excess of oxygen, increased the output of the mammalian heart. In greater amounts, however, the action was depressant. Some doubt as to the correctness of the conclusions arrived at by these authors, namely, that carbon dioxide in proper amount is beneficial to cardiac function, must result from the observations to be reported in this paper. Studying the hearts of frogs and turtles, Jerusalem and Starling found that carbon dioxide was uniformly depressant. In a following paper we have criticised their methods of observation of the mammalian heart, and on the basis of the results here reported believe we have shown that carbon dioxide is likewise a depressant for the mammalian heart.

In addition to the observations on vascular muscle there are also here reported parallel observations on intestinal muscle. The latter, except when rhythmically active, is caused to contract by an amount of carbon dioxide which produces the opposite effect, namely, relaxation, in vascular muscle from the same animal. When, however, the intestinal muscle is exhibiting rhythmicity, it reacts to carbon dioxide exactly as does vascular muscle (relaxation). Bayliss and Starling⁵ have observed the same response in rhythmic loops of gut when the blood supply is shut off. No explanation of this phenomenon is at hand, and it appears the more difficult to explain when it is seen that the vascular muscle always reacts to carbon dioxide with relaxation whether exhibiting rhythmicity or not.

In structure the wall of the intestine differs from that of a blood vessel in containing sympathetic nerve cells. Both tissues have anatomically similar contractile elements, and both contain sympathetic nerve fibres. It has been suggested that the presence of nerve cells in the wall of the intestine and their absence in the wall of the blood vessels may explain the difference in reaction. Obviously the alternative explanation must assume an inherent difference in irritability in anatomically similar structures, *i. e.*, the nerve fibre and contractile cell in the vessel and gut. We have no justification for

⁴ JERUSALEM and STARLING: *Journal of physiology*, 1910, xl, p. 279.

⁵ BAYLISS and STARLING: *Proceedings 4th International Physiological Congress*, Cambridge, 1898 (*Journal of physiology*, 1898, xxiii, Supplement, p. 34).

such an assumption, nor is the former explanation satisfactory when applied to the reaction of the intestinal muscle. The data gathered in this paper do not warrant, however, extended discussion of this question.

METHODS.

Ring preparations of vessel or gut about 5 mm. in length were suspended in a small water-jacketed glass chamber, the temperature of which could be regulated. The chamber was provided with an inlet tube at the top for the entrance of gas; at the bottom a small opening permitted the escape of gas, and also the attachment of the muscle preparation to the recording lever.

The gas mixtures were prepared in a calibrated gasometer and transferred to gas bags. From the latter the gas was led by rubber tubing to the muscle chamber after being washed by bubbling through water. A screw clamp controlled a constant rate of flow for the different gas mixtures acting on the preparation under observation.

EXPERIMENTAL.

Arterial muscle of cold blooded animal. — Two preparations were studied, both of which were taken from the turtle. The first of these was from the right descending aorta. It reacted with marked loss of tone when submitted to a pure atmosphere of carbon dioxide. The tone was promptly recovered or improved when the carbon dioxide was replaced by an atmosphere of oxygen or of air. The second preparation was from one of the aortic branches in the neighborhood of the bulbus. The response of this preparation was as follows:

Gas mixture.*	Tone of muscle.	Gas mixture.	Tone of muscle.
O ₁₀₀	Improved	O ₂₀ H ₈₀ . .	Improvement
C ₁₀₀	Sharp loss	O ₁₀₀	Slight improvement
O ₂₀ H ₈₀ . . .	Slight improvement	C ₅ O ₉₅ . . .	Loss
O ₁₀₀	Sharp improvement	O ₁₀₀	Improvement
C ₁₀ O ₉₀	Loss

The record from this preparation showed that as little as 5 per cent of carbon dioxide is sufficient to produce a loss of tone. That such

* Here and in all subsequent references to gas mixtures the sub-figures represent parts in one hundred. The letter C is used to represent carbon dioxide.

loss of tone is not due to the reduction in the amount of oxygen is indicated by the fact that after the tone has been depressed by 10 per cent of carbon dioxide in an oxygen atmosphere, it is promptly recovered by 20 per cent of oxygen in an hydrogen atmosphere.

Both of these preparations exhibited independent rhythmic contractions.

Venous muscle from cold-blooded animal.—Three preparations were investigated, each of which was taken from the sinus region in the turtle. They all showed contractions as well as rhythmic tone changes. In the first both pulsations and tone waves were abolished by carbon dioxide, a condition which was followed by marked loss of tone. In oxygen pulsation, tone waves and tone were restored. In the second preparation the following effects were observed:

Gas mixture.	Tone of muscle.	Pulsations.	
		Amplitude.	Rate.
O ₁₀₀
N ₈₀ O ₂₀ . . .	No change
N ₆₀ O ₂₀ C ₂₀ . .	Loss
N ₄₀ O ₂₀ C ₄₀ . .	Further loss	Decrease	Decrease
O ₁₀₀	Improvement	Increase	Increase
C ₁₀₀	Sharp loss	Pulsations ceased	
O ₁₀₀	Recovery	Recovery	Recovery
N ₈₀ O ₂₀ . . .	No change	No change	No change
N ₆₀ O ₂₀ C ₂₀ . .	Loss	Decrease	?
N ₈₀ O ₂₀ . . .	Recovery	Increase	No change
N ₆₀ O ₂₀ C ₂₀ . .	Loss
N ₈₀ O ₂₀ . . .	Recovery
N ₄₀ O ₂₀ C ₄₀ . .	Loss	Pulsations ceased	
N ₉₆ O ₄ . . .	Improvement	Improvement	Improvement
O ₁₀₀	Complete recovery	Weak	Slow

It will be noted that in this preparation the effect of carbon dioxide (20 per cent in an atmosphere of oxygen) was to depress both the tone and amplitude of contraction; further, that after the tone and contractions were abolished by the mixture N₄₀O₂₀C₄₀ a considerable improvement followed the use of mixture N₉₆O₄. Thus the depression caused by carbon dioxide in the presence of oxygen was in part abolished by as little as 4 per cent of oxygen in an atmosphere

of nitrogen. Part of the record from the third preparation studied is reproduced in Fig. 1. The data from this preparation are as follows:

Gas mixture.	Tone of muscle.	Pulsations.	
		Amplitude.	Rate.
Room air.			
C ₁₀₀	Loss	Pulsations ceased	
O ₁₀₀	Recovery	Increased	Recovery
H ₁₀₀	Slow loss
C ₁₀₀	Loss	Pulsations ceased	
O ₁₀₀	Recovery	Recovery	Recovery
C ₁₀ O ₉₀ . . .	Loss
O ₂₀ H ₈₀ . . .	Recovery
C ₅ O ₉₅	Loss
O ₂₀ H ₈₀ . . .	Improvement
O ₁₀₀	Further improvement

This brings out clearly that hydrogen is a relatively inert gas, and that a loss of tone produced by 10 per cent of carbon dioxide in an atmosphere of oxygen may be recovered by 20 per cent of oxygen in an atmosphere of hydrogen. In other words, the tone or loss of tone is dependent upon the carbon dioxide in the gas mixture. In this preparation 5 per cent of carbon dioxide was sufficient to depress the tone of the muscle.

Arterial muscle from warm-blooded animal.—This tissue loses tone in an atmosphere of carbon dioxide and regains its tone in an atmosphere of oxygen. After this fact was established the minimum effective amount of carbon dioxide was determined as given below. The preparation used was from a cat's thoracic aorta kept during observation at a temperature of 38.5° C. Chloretone was used for anæsthesia.

Gas mixture.	Tone of muscle.	Gas mixture.	Tone of muscle.
O ₈₀ N ₂₀	Increased	O ₈₀ C ₂₀	Decreased
O ₈₀ C ₂₀	Decreased	O ₈₀ N ₂₀	Increased
O ₈₀ N ₂₀	Increased

Accordingly 20 per cent carbon dioxide is required to cause this muscle to lose tone. This statement is based on the observations of a number of other preparations which failed to be effected by carbon dioxide in lesser amounts, but which nevertheless relaxed in amounts greater than 20 per cent.

Venous muscle from warm-blooded animal.—Four preparations were studied, one from the portal vein of rabbit and three from portal vein of cats.

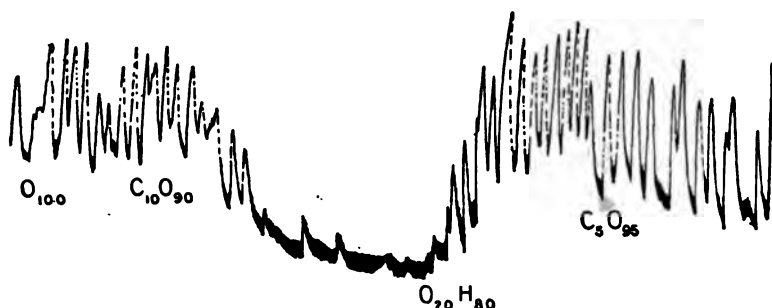


FIGURE 1.—Four fifths the original size. Ring preparation from sinus venosus of terrapin. The sub-figures to the letters indicate parts in one hundred of the gas. Drum moved 1 centimetre in three and one-half minutes.

Preparation I from portal vein of rabbit kept at a temperature of $35-35.5^{\circ}$ C. gave the following results:

Gas mixture.	Tone of muscle.	Gas mixture.	Tone of muscle.
Oxygen	Improved	Oxygen	Improved
Carbon dioxide . .	Relaxed	Carbon dioxide . .	Relaxed
Oxygen	Improved	Oxygen	Improved
Carbon dioxide . .	Relaxed	Carbon dioxide . .	Relaxed
Oxygen	Improved	Oxygen	Improved
Carbon dioxide . .	Relaxed

Throughout the greater part of this record irregular rhythmic tone changes are apparent.

Preparation II from portal vein of cat kept at a temperature of 39° C. gave the following results:

Gas mixture.	Tone of muscle.	Gas mixture.	Tone of muscle.
$N_{80}O_{20}C_{20}$	Relaxed	C_{100}	Relaxed
O_{100}	Improved	O_{100}	Improved

The record shows rhythmic tone changes during the first period of exposure to oxygen.

Preparation III from portal vein of cat gave the following results:

Effect of Carbon Dioxide and Oxygen upon Muscular Tone. 53

Gas mixture.	Tone of muscle.	Temperature variation.	Duration of exposure.
Room air.	Improved	40, 38.5, 37.5, 38.5, 39	12 min.
Carbon dioxide	Relaxed	39, 38, 36.5, 36	10 min.
C ₈ O ₉₂	Slight improvement	36, 35.5, 36, 36	8 min.
C ₂₀ O ₈₀	No effect	36, 35.5, 36.5	6 min.
C ₂₀ O ₈₀	Relaxed	36.5, 37, 36.5	6 min.

No rhythmic tone changes were noted. Although the temperature fluctuated slightly in the several periods, it did not interfere with

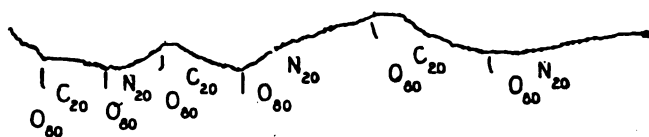


FIGURE 2. — Ring preparation from portal vein of cat. Temperature constant at 40° C. The sub-figures to the letters indicate parts in one hundred of the gas. Drum moved 1 centimetre in one and two-tenths minutes.

the reaction of the muscle to change in its gaseous environment. It will be noted that the loss of tone produced by carbon dioxide is in part recovered when a mixture replacing the greater part of the carbon dioxide with oxygen is substituted. Such recovery is, however, incomplete, and when the carbon dioxide is again increased in amount, this improvement disappears.

Preparation IV from the portal vein of cat gave the record which is reproduced in Fig. 2. Throughout the observation the temperature was maintained at 40° C. The following data are obtained from this record:

Gas mixture.	Tone of muscle.	Gas mixture.	Tone of muscle.
O ₈₀ C ₂₀	Relaxed	O ₈₀ N ₂₀	Improved
O ₈₀ N ₂₀	Improved	O ₈₀ C ₂₀	Relaxed
O ₈₀ C ₂₀	Relaxed	O ₈₀ N ₂₀	Improved

No rhythmicity was apparent in this preparation. It is apparent that the relaxation was due entirely to the carbon dioxide since the tone of the muscle improved when nitrogen was substituted without changing the amount of oxygen supplied to the tissue.

Intestinal muscles from cold-blooded animal. (A) *When arrhythmic.* — Ten such preparations were studied. They were all taken from frogs

in the months of January, May, October, and December. An example of the response obtained is given in the record reproduced in

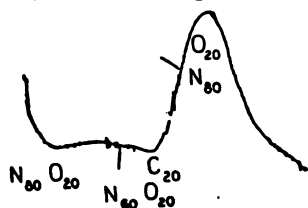


FIGURE 3.—Ring preparation from prepylorus of frog. The sub-figures to the letters indicate parts in one hundred of the gas. Drum moved 1 centimetre in one and two-tenths minutes.

Fig. 3, which shows the effect of 20 per cent of carbon dioxide in causing contraction of the muscle. The balance of the data gathered from these experiments may be presented in condensed form as follows:

- Fundus of stomach one preparation.
- Prepylorus of stomach two preparations.
- Pylorus of stomach four preparations.
- Duodenum of stomach one preparation.

Gas mixture.	Effect on muscle and number of times observed.		
	Contract.	Relax.	No effect.
O ₁₀₀	0	11	0
H ₁₀₀	0	1	0
C ₁₀₀	13	0	0
Air	0	3	0
N ₈₀ O ₂₀	0	7	4
N ₇₀ O ₂₀ C ₁₀	1	0	4
N ₇₅ O ₂₀ C ₅	0	0	1
N ₉₀ C ₁₀	1	0	0
N ₆₀ O ₂₀ C ₂₀	1	0	1
N ₈₀ C ₂₀	0	0	1
N ₆₀ C ₄₀	1	0	0
O ₉₅ C ₅	0	1	1
O ₈₀ C ₂₀	1	0	0

It is evident from this that the predominating effect of carbon dioxide is to cause contraction of the muscle when in an arrhythmic state. These results are in sharp contrast to those which follow:

(B) *When rhythmic.*—Fourteen such preparations were studied. As in the case of the arrhythmic preparations they were taken from frogs in the months of October, November, and December. The seasonal condition of the frog thus appears not to be a factor in the difference in behavior of similar tissues to given gas mixtures. This conclusion is further substantiated by three preparations which developed or lost their rhythmicity during the observations. When arrhythmic

they behaved as those above discussed; when rhythmic, they exhibited opposite reactions and fell into the group now under consideration.

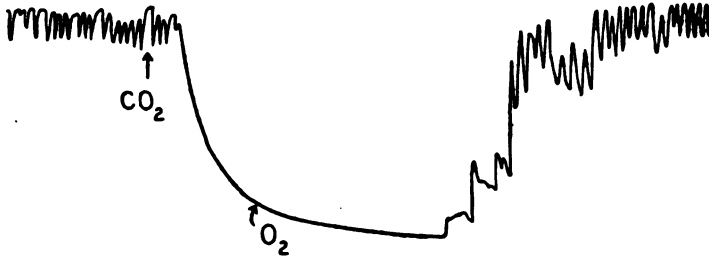


FIGURE 4. — Ring preparation from duodenum of frog. Drum moved 1 centimetre in one and two-tenths minutes.

Fig. 4 is given as examples of this group. The remaining observations may be condensed as before:

Fundus of stomach two preparations.
 Prepylorus of stomach one preparation.
 Pylorus of stomach two preparations.
 Duodenum of stomach seven preparations.

Gas mixture.	Effect on muscle and number of times observed.		
	Contract.	Relax.	No effect.
O ₁₀₀	29	0	0
H ₁₀₀	0	6	0
C ₁₀₀	0	12	0
Air	1	0	0
O ₉₀ C ₁₀	0	7	0
O ₉₀ H ₁₀	2	0	2
O ₉₅ C ₅	0	8	0
O ₅₀ H ₅₀	0	0	1
H ₈₀ O ₂₀	2	0	0
O ₈₀ C ₂₀	0	5	0

It will be noted here that these rhythmic preparations appear more sensitive than those which are arrhythmic; 5 per cent of carbon dioxide in oxygen being sufficient to inhibit rhythmicity and to depress tone. While this is especially true of even small amounts of carbon dioxide, it is further apparent that the tissue loses tone and rhythmicity in the absence of oxygen.

Intestinal muscle from warm-blooded animal. — Rhythmicity was observed in but one of the seven preparations studied (oesophagus). In one other preparation rhythmicity was present for a short time at the beginning of the experiment, but disappeared before any observations were made. Probably, therefore, the experimental conditions were imperfectly adapted to the tissue investigated.

The preparations were obtained as follows:

Duodenum of cat one preparation.
 Duodenum of kitten four preparations.
 Gastric cardia of kitten one preparation.
 Oesophagus of kitten one preparation.

The observations may again be condensed as follows:

Gas mixture.	Effect on muscle and number of times observed.		
	Contract.	Relax.	No effect.
O ₁₀₀	0	5	0
C ₁₀₀	9	0	0
O ₈₀ N ₂₀	0	5	1
O ₈₀ C ₂₀	4	0	1
O ₈₀ C ₁₀ N ₁₀	1	0	1
Air	0	2	0

These figures represent the arrhythmic preparations and show that carbon dioxide when in amount sufficient to produce effect invariably produces a tone contraction.

The behavior of the oesophagus preparation (rhythmic) was as follows:

Rhythmic or not.	Gas mixture.	Tone change.
No	Air	Relaxation
No	O ₈₀ C ₁₀ N ₁₀	Contraction
Yes	O ₈₀ N ₂₀	Further contraction
Yes	O ₈₀ C ₁₀ N ₁₀	Relaxation
Yes	O ₈₀ N ₂₀	Contraction
Yes	O ₈₀ C ₂₀	Relaxation
Yes	O ₈₀ N ₂₀	Contraction
Yes	O ₈₀ C ₁₀ N ₁₀	Relaxation

Too much emphasis should not be put on this single experiment. In connection with the other results from warm-blooded intestinal

muscle, however, it brings this class of tissue in line with the observations made upon similar preparations from cold-blooded animals. It suggests, further, that the peculiar dual behavior of this particular type of muscle in respect to its reaction to gas mixtures may possibly be explained by the metabolic state of the contractile substance. For, as may be noticed, the preparation just after removal from the body and perhaps before its temperature was brought back to normal, exhibited no rhythmicity, and in this condition it was stimulated to tone contraction by carbon dioxide. Later in the experiment, while exhibiting rhythmicity, the same percentage of carbon dioxide twice produced distinct relaxation. Mention has already been made that three preparations classed as "rhythmic" from the intestine of frogs, displayed the same phenomenon. These frogs had been kept in a cool place, and it may well be that their metabolic processes had been depressed by the cold as were those of the cat's oesophagus preparation by the cooling incident to its transfer from the body to the warm chamber. Since, however, such observations were incidental and no effort was made to control the conditions in respect to the metabolic state of the muscle, further discussion of this point is profitless without more experimental data.

CONCLUSIONS.

1. The muscular tissue in the walls of the blood vessels and alimentary tract is capable of independent rhythmic movement.
2. Carbon dioxide does not appear to be *directly* beneficial to this tissue, except in the case of intestinal muscle when arrhythmic.⁷

⁷ This statement does not negate the possibility of an indirect benefit, as, for example, in facilitating the circulation of blood by producing vascular relaxation and, if the intestinal muscle is arrhythmic, by squeezing blood out of the venous plexuses of the gut. It is obvious that the circulation may react in the converse sense when the carbon dioxide is reduced in amount, thus offering the hypothesis of a peripheral mechanism of economy which would shunt blood from inactive to active tissues. Kaya and Starling (Journal of physiology, 1909, xxxix, p. 346) have shown that the administration of increased amounts of carbon dioxide to the spinal animal does not produce the typical rise of arterial pressure. There is instead a slight fall of pressure. This observation indicates that in the intact animal carbon dioxide produces in the medullary centres an over-compensation of the unfavorable effects produced in the periphery. On the basis of these facts we may properly ask if the administration of carbon dioxide in cases of surgical

3. Carbon dioxide in minimal effective amounts always relaxes vascular muscle. If the muscle is exhibiting rhythmicity, the rhythmicity is abolished or depressed.

4. Oxygen is essential to rhythmicity in vascular muscle, and also to its maintenance of tone.

5. Intestinal muscle, if *rhythmic*, responds to carbon dioxide and oxygen as does vascular muscle.

6. Intestinal muscle, on the other hand, if *arrhythmic*, responds to carbon dioxide with contraction and to oxygen with relaxation.

7. This difference in behavior of rhythmic and arrhythmic preparations of intestinal muscle is tentatively explained as due to the metabolic state of the tissue. Nevertheless this explanation does not reconcile anatomical structure and physiological activity in intestinal and vascular muscle, since the latter tissue reacts consistently to carbon dioxide or oxygen regardless of whether it is rhythmic or arrhythmic. Hence the necessity of considering anatomically similar contractile tissues capable of divergent physiological reactions.

8. The kind of reaction produced by carbon dioxide and oxygen is the same for all percentages; the amount of reaction varies directly with the amount of gas.

shock, as advised by Henderson (Johns Hopkins Hospital bulletin, 1910, xxi, p. 1), is an advantageous form of stimulation, since the peripheral effect of such stimulation weakens the central (medullary) effect? If a stimulus is advisable in this condition, it ought, if possible, to be of direct as well as indirect benefit to vascular tone.

THE COMPARATIVE SENSITIVENESS OF BLOOD PRESSURE AND INTESTINAL PERISTALSIS TO EPINEPHRIN.

By R. G. HOSKINS AND C. W. McCLURE.

[From the Laboratory of Physiology of the Starling-Ohio Medical College.]

MUCH of the late research on the physiology of the adrenals is based more or less directly upon Elliott's brilliant paper published in 1905.¹ This investigator established conclusively the fact that there is a peculiar, intimate relationship between the adrenal glands and the sympathetic nervous system. His experiments were based upon the injections of epinephrin. There was within the non-toxic limits however, little or no restriction as to quantity.

That a drug may produce very different effects, depending upon the quantity used is an elementary principle of pharmaco-dynamics. In the case of epinephrin a reversal of reaction with change of quantity has in several instances been specifically noted. Intestinal activity is strongly depressed by maximal quantities of the drug, but increased by minimal quantities.² Stewart has recently reported a similar reversal of effect in uterine tissue.³ Desbouis and Langlois have observed that larger quantities cause constriction of the pulmonary blood vessels, while smaller quantities cause dilatation.⁴ Similarly, systemic blood pressure is elevated or depressed according to the quantity injected.⁵ It by no means follows, therefore, that because the injection of a large dose of epinephrin produces a certain result a similar effect is produced by the quantity normally present in the circulating blood. This fact leaves open the question to what

¹ ELLIOTT: *Journal of physiology*, 1905, xxxii, p. 401.

² HOSKINS: *this Journal*, 1912, xxix, p. 363.

³ STEWART: *Journal of experimental medicine*, 1912, xv, p. 547.

⁴ DESBOUIS et LANGLOIS: *Comptes rendus de la Société de Biologie*, 1912, lxxii, p. 674.

⁵ HOSKINS and McCLURE: *Archives of internal medicine*, 1912, in publication.

extent Elliott's results are applicable to the normal functions of the adrenals. In a communication published elsewhere⁵ data have been reported which indicate that adrenal secretion cannot be an immediate factor in vasomotor tonus. The evidence in brief is that the adrenals do not produce more than one fourth enough secretion to affect blood pressure, — that the effect of injections gradually transcending physiologic concentration is primarily *depression*, and that approximately five times as much of the drug is required to give a minimal sustained rise as to produce this primary effect. The adrenals produce at most, therefore, only approximately one twentieth enough secretion to exert a minimal *pressor* influence.

Another method of investigating the applicability of Elliott's results to normal conditions suggested itself, — namely, to compare the simultaneous effects of epinephrin upon blood pressure and peristalsis. That larger doses of the drug raise blood pressure and depress peristalsis is well known. If it were shown that peristalsis is the more sensitive of the two, such a finding would indicate that epinephrin is not an immediate factor in maintaining tonus in the sympathetic nervous system, because an organism obviously could not utilize a mechanism to maintain vascular tonus which at the same time would materially depress peristalsis. The results of an investigation of the comparative sensitiveness of blood pressure and peristalsis to epinephrin are embodied in this paper. Previous investigations had shown that adrenalin in sufficiently great dilution can be injected continuously into the femoral vein without demonstrable effect upon blood pressure.⁵ As the quantity is gradually increased, then, the first effect to appear would supposedly be an increment of the physiologic effect of normally circulating epinephrin.

In our experiments dogs were etherized and cannulas inserted into the carotid artery and femoral vein and a balloon into the small intestine. Then, while simultaneous records of carotid blood pressure and peristalsis were being secured, epinephrin in varying quantities and for different periods of time was injected into the femoral vein. Thus epinephrin discharge from the adrenal glands was simulated. Blood pressure was recorded by means of an ordinary mercury manometer and float. Peristalsis was recorded by connecting the rubber balloon in the small intestine with a modified Albrecht recorder. A cylinder about 2 cm. in diameter was connected by rubber tubing

with the balloon. The whole system was filled with water at 35 cm. pressure. In the cylinder floated a cork connected by a light upright with a writing lever magnifying some twenty times. Care was used in inserting the balloon to avoid as much as possible visceral irritation which might cause inhibitory reflexes to the intestines. As a matter of fact little difficulty was experienced in getting satisfactory long-continued records of peristalsis and of fluctuations in intestinal tonus. The apparatus, in fact, was over-sensitive in that damping was required to prevent an undue prominence of respiration waves in the records.

The epinephrin used was Parke, Davis, and Co.'s "adrenalin." This was diluted with Ringer's solution to a concentration of 1:100,000 to 1:500,000, as the conditions of each experiment demanded. The purpose in each case was to use a concentration such that effects were produced without the injection of significant quantities of fluid, but sufficiently dilute that the rate of inflow could readily be controlled. The fluid was injected from a burette graduated to tenths of a cubic centimetre. The flow was controlled by an ordinary glass stopcock. The injections produced their first effect in about fifteen seconds. Series of injections of thirty seconds each were made at short intervals until the minimal effective rate of inflow was determined for each individual animal. Then injections for this and longer periods were made at the minimal and gradually increasing rates. With such procedure there was no demonstrable loss of sensitiveness during the course of the experiment.

In all, eight experiments were made. The results secured were consistent throughout. Peristalsis was brought to a stand-still, and extreme intestinal dilatation produced by quantities inadequate to cause a minimal sustained rise of pressure. Fig. 1 shows typical records of the effects of minimal and supraminimal doses. With injections at the rate causing minimal unmistakable depression of tonus and of peristalsis blood pressure was either absolutely unaffected or else slightly depressed. Long before a minimal sustained hypertension occurred intestinal activity was brought to a complete stand-still.

The data of this paper offer further support to a hypothesis proposed elsewhere.⁵ The adrenal glands under ordinary conditions do not produce sufficient secretion to exert a tonic influence upon the

sympathetic system. This is shown both by direct injection and by extirpation experiments. So far as the relationship between the adrenals and the sympathetic system is concerned, it is merely a mechanism to promote muscular efficiency in times of special stress, while the asthenia resulting from adrenal extirpation is due to an interference with the nutrition of the muscular tissues. In fact, Biedl's researches

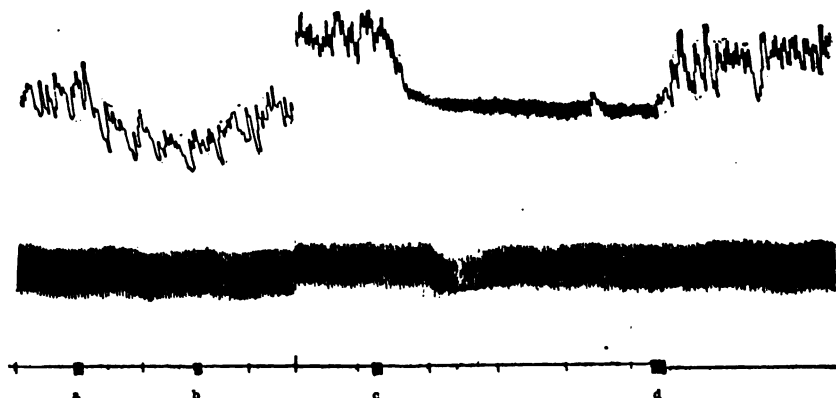


FIGURE 1. — One half the original size. Simultaneous effects of adrenalin upon intestinal peristalsis (upper tracing) and carotid blood pressure of dog (16.1 kilos). From *a-b* injection of 0.8 c.c. adrenalin, 1: 200,000, in Ringer's solution (1 minute); from *c-d*, 2 c.c. (two minutes). "Time," thirty seconds. (Difference in height of peristalsis records in two cases due to readjustment of recorder. Secondary waves in peristalsis record due to respiration.)

indicate, though inconclusively, that the asthenia resulting from adrenal extirpation is due to the loss of cortical tissue which has no known relationship to the sympathetic system. The hypothesis proposed is based upon the facts that under conditions demanding extreme muscular effort there is a greatly augmented epinephrin discharge from the adrenal glands.⁶ Injections of maximal quantities of epinephrin lead to a depression of alimentary activity, strengthened heart beat, and extreme vascularity of the central nervous system, lungs, liver, and kidneys, with ischemia of the skin, mucous membranes, quiescent muscle, etc.⁷ Assuming that the local vasodilator mechanisms be efficient in active muscle to overcome the constricting effect

⁶ CANNON and DE LA PAZ: this Journal, 1911, xxviii, p. 64; CANNON and HOSKINS: *Ibid*, 1911, xxix, p. 274.

⁷ FALTA and PRIESTLY: *Berliner klinische Wochenschrift*, 1911, xlviii, p. 2102.

of circulating epinephrin, — as they obviously must be, — an adaptive distribution of blood would be provided to the structures necessarily active, — *i. e.* the motor, eliminative, and respiratory systems, — while tissues not involved would remain quiescent with a minimal blood supply. Hepatic vascularity would not only promote excretion, but also make freely available any glycogen stored in the liver. The well-known stimulation of the respiratory centre by larger doses of epinephrin is also significant in this connection. From their data regarding the distribution of the blood under the influence of large doses of adrenalin, Falta and Priestly have deduced a theory that a function of the chromaffin tissue is to secure distribution of blood favorable to ordinary bodily activities with a minimal cardiac effort. The fact, however, that intestinal depression supervenes with a minimal dosage of epinephrin before the vasomotor system is affected, as well as the general principle that effects of maximal doses have no valid bearing on the problem of the functions of the adrenals under ordinary conditions, would seem to disprove their theory.

SUMMARY.

Simultaneous records of peristalsis and blood pressure show that intestinal depression is caused by injections of epinephrin decidedly smaller than those requisite to cause increase of blood pressure. Normal vasomotor tonus, therefore, is not dependent upon a minimal stimulating effect of circulating epinephrin. The chromaffin tissue probably has a function of causing in times of special stress an adaptive distribution of the blood favorable to extreme muscular effort.

THE EFFECT OF CARBON DIOXIDE ON THE ISOLATED HEART.

By C. S. KETCHAM, J. T. KING, JR., AND D. R. HOOKER.¹

[From the Physiological Laboratory of the Johns Hopkins University.]

INTRODUCTION.

IN the preceding paper observations are reported which indicate that carbon dioxide must be regarded as detrimental to cardiovascular tissue in that it relaxes tone and abolishes contractile rhythm. As a consequence of these results it appeared of importance to repeat the work of Jerusalem and Starling.² These authors found that carbon dioxide was uniformly injurious to the cold-blooded heart. But in the case of the mammalian heart a certain amount of carbon dioxide, approximately 5 to 8 per cent, was beneficial rather than injurious to activity. *A priori* it seemed to us improbable that such difference in behavior of cold and warm blooded hearts should exist, and we inclined to the belief that the explanation was to be found in the methods of investigation rather than in inherent differences in the hearts themselves. This in fact proved to be the case.

EXPERIMENTAL.

The gas mixtures, the effects of which were to be investigated, were prepared in a calibrated gasometer and at once transferred to rubber gas bags. The mixtures so prepared were then bubbled through the perfusion fluid until saturation was assumed to be complete. In each experiment, as will be seen by reference to the tables, the amount of oxygen supplied to the heart was maintained constant, and the control mixture simply substituted nitrogen for carbon dioxide.

¹ Mr. H. SALTZSTEIN collaborated in a part of the work.

² JERUSALEM and STARLING: *Journal of physiology*, 1910, xl, p. 279.

The Effect of Carbon Dioxide on the Isolated Heart. 65

The cold-blooded heart (terrapin) was perfused with Ringer's solution under constant pressure (2.5 cm.) from two flasks, the contents of the one being saturated with oxygen plus carbon dioxide, and that of the other with oxygen plus nitrogen. Our results entirely confirm the work of Jerusalem and Starling and most of the earlier investigators. The data from this series of experiments are collected in the following table (Table I). The values given are relative only, but show distinctly the effect upon the heart of carbon dioxide.

TABLE I.
DATA FROM COLD-BLOODED HEART.

Experi- ment.	Perfusion fluid saturated with	Rate.	Amplitude ventricular contraction.	Ventricular tone.	Auricular tone.	Ventric- ular output.
	per cent.					c. c.
I	O ₂ 90, N 10	50	9.0	64	19	36.0
	O ₂ 90, CO ₂ 10	50	3.0	50	6	8.0 ^a
	O ₂ 90, N 10	44	9.0	53	12	27.0
	O ₂ 95, CO ₂ 5	37	3.5	40	0	4.5 ^a
	O ₂ 90, N 10	37	8.0	40	0	27.0
II	O ₂ 95, N 5	45	4.5	93	45	..
	O ₂ 95, CO ₂ 5	45	4.5	79	25	22.0 ^a
	O ₂ 95, N 5	44	8.0	82	36	31.5
	O ₂ 90, CO ₂ 10	41	4.0	80	23	13.5 ^a
III	O ₂ 97½, N 2½	34	10.0	85	26	..
	O ₂ 97½, CO ₂ 2½	39	8.0	80	23	..
	O ₂ 97½, N 2½	43	10.0	82	27	..
	O ₂ 97½, CO ₂ 2½	43	10.0	72	18	..
	O ₂ 97½, N 2½	42	11.0	72	23	..

Our technique in the study of the cold-blooded heart was the same as that of Jerusalem and Starling, and the results were the same. In the case of the mammalian heart Jerusalem and Starling used a modification of the method first used by Martin in this laboratory in 1881.

^a Auricular contraction stopped.

The blood expelled by the left ventricle is led through an aortic cannula to a mercury valve, maintaining a constant arterial pressure, from which it is returned to the right heart. The heart is thus isolated *with the lungs*, and aeration of the blood is accomplished with artificial respiration. By using selected gas mixtures for the latter they were able to vary the percentage amount of carbon dioxide in the circulating blood. Their results show that with an optimum percentage (2 to 8) of carbon dioxide in the respired air, both systolic and diastolic volume of the heart are increased, but that diastolic volume is increased the more, so that the unit output is increased. Together with this relaxation there is a slowing in the rate of beat, but the latter does not compensate the increased discharge per beat, so that there is an actual increase in the minute volume of the heart.

It was assumed from the data presented in the preceding paper that in such a heart-lung preparation carbon dioxide might cause a loss of tone in the pulmonary vascular bed, and this in turn might influence the work of the left ventricle. In a word, the diastolic volume would increase proportionally to the increase of inflow pressure, and so long as the heart could carry the increased load, the effect would appear as an increase of working capacity, without being an evidence of the beneficial effect of carbon dioxide.

To test this point of view cats' hearts were isolated according to Martin's second method, and fed with Locke's solution made up of NaCl 0.9 per cent, CaCl_2 0.03 per cent, KCl 0.03 per cent, dextrose 0.1 per cent, and NaHCO_3 0.02 per cent, at a pressure of 60 mm. Hg. The perfusion apparatus was arranged with two flasks. In one of these the solution was saturated with oxygen plus carbon dioxide, and in the other with oxygen plus nitrogen. By this means it was possible to investigate the effect of carbon dioxide on the heart independent of possible changes in the pulmonary circulation. The record was obtained by anchoring the heart to the thoracic wall, and leading threads from right auricle and ventricle to writing levers properly weighted.

Three experiments were performed with consistent results. Fig. 1 is reproduced from a part of the record of one of them. It will be seen that tone, amplitude of contraction, and rate are depressed under the influence of carbon dioxide. Upon changing back to the oxygen-nitrogen solution the functional capacity of the heart is restored. The



7% N	CO, 3%	N 7%	CO, 7%	N 7%	CO, 3%	N 7%
93% O	O 97%	O 93%	O 93%	O 97%	O 97%	O 93%
Rate 125	T. & P. same	Rate 130	Rate 109	Rate 125	Rate 110	Rate 125
Temp. 35°	T. & P. same	T. & P. same	Pres. 65	Pres. 70	T. & P. same	T. & P. same
Pres. 70 mm.			Temp. 35°	Temp. 34°		

FIGURE 1.—One half the original size. Record from isolated cat's heart to show the depressant action of carbon dioxide. First line, contraction of right ventricle. Second line, contraction of right auricle. Third line, time in minutes. Fourth line, signal indicating change of gas-saturated Locke's solution. The figures are given as parts of oxygen, carbon dioxide, and nitrogen in one hundred. The apparent rapid loss of tone shown at the beginning of the auricular record is due to stretching by the lever weight. The coronaries were perfused at a pressure of 60 mm. Hg.

data gathered from these experiments are presented in Table II. The values given, except the rate, are relative, but, as in the case of the cold-blooded heart, they show the distinctly depressant action of carbon dioxide.

These results are in complete agreement with those obtained by Jerusalem and Starling, and by ourselves from the cold-blooded animal. They are not, however, in agreement with the work of Jerusalem and Starling on the mammalian heart. We find that the heart rate is slowed by carbon dioxide. This is in accordance with the results of Henderson,⁴ Starling, and others. Reference to the table will show that our results in this respect are not entirely uniform. This, we believe, is due entirely to variations in the temperature. In the third experiment, in which special attention was directed to this point, the results are consistent, and in agreement with the results of other observers.

The amplitude of both auricular and ventricular beats is distinctly lessened by carbon dioxide in amounts as small as 3 per cent. With 7 per cent of the gas the effect is still more pronounced.

The tone of the auricular and ventricular muscle, as measured from a given base line, shows the same depressant effect of carbon dioxide. As in the case of amplitude of contraction, 3 per cent of the gas is effective, but the results are much more marked with 7 per cent. The registration of the tone of the muscle offered considerable technical difficulty in the choice of the lever load. On the whole, however, the results are clear and definite.

Jerusalem and Starling state that their method of studying the mammalian heart is of advantage in that approximately normal blood is fed the heart, and that in this blood carbon dioxide would be present under normal conditions. To meet this possible objection to our work we attempted to perfuse rabbits' hearts with defibrinated sheep's blood, but were entirely unsuccessful. It appeared on consideration, however, that this could not be regarded as a valid criticism. The heart would undoubtedly beat more strongly when fed with blood than with a salt solution. But the first portion of carbon dioxide to affect the heart would be that in solution. Hence we are inclined to believe that our method gives a satisfactory test.

In order to ascertain what, if any, influence the presence of car-

⁴ HENDERSON: *this Journal*, 1906, **xii**, p. 120.

TABLE II.
DATA FROM MAMMALIAN HEART.

Experiment.	Perfusion fluid saturated with	Rate.	AMPLITUDE.		TONE.	
			Auricle.	Ventricle.	Auricle.	Ventricle.
I	per cent O ₂ 93, N 7	43	2.0	2.5	1.9	7.0
	O ₂ 93, CO ₂ 7	66	0.0	1.0	2.3	7.0
	O ₂ 93, N 7	61	0.0	3.0	2.	7.0
	O ₂ 93, CO ₂ 7	60	0.0	1.0	1.8	6.8
	O ₂ 93, N 7	60	0.0	2.5	1.5	6.8
	O ₂ 93, CO ₂ 7	54	0.0	1.0	0.9	6.5
	48	0.0	0.6	5.7
	O ₂ 93, N 7	48	2.2	4.5
	O ₂ 93, CO ₂ 7	43	0.7	4.7
	O ₂ 93, N 7	43	2.0	4.7
II	O ₂ 93, N 7	72	0.5	2.0	5.9	8.2
	O ₂ 97, CO ₂ 3	60	0.4	1.9	5.4	7.6
	O ₂ 93, N 7	54	0.7	2.1	5.4	7.8
	O ₂ 93, CO ₂ 7	50	0.4	1.4	5.4	7.5
	O ₂ 93 N 7	37	1.9	2.4	5.2	7.6
III	O ₂ 93, N 7	125	1.8	2.5	2.0	7.0
	O ₂ 97, CO ₂ 3	112	0.8	2.3	1.9	6.8
	O ₂ 93, N 7	130	1.0	2.4	1.2	6.8
	O ₂ 93, CO ₂ 7	102	0.0	1.5	0.6	5.8
	O ₂ 93, N 7	125	1.0	2.3	0.8	6.6
	O ₂ 97 CO ₂ 3	110	0.4	2.0	0.6	6.5
	O ₂ 93, N 7	125	0.5	2.0	0.5	6.7
	105	0.5	2.1	2.0	6.3
	O ₂ 93, CO ₂ 7	72	0.0	1.5	2.0	5.9
	O ₂ 93, N 7	84	0.3	2.2	2.0	6.2

bon dioxide in the circulating fluid would have upon the calibre of the pulmonary vessels and consequently upon the resistance to the flow of blood to the left heart, we perfused the lungs of terrapins and rats with solutions saturated with known gas mixtures.

In the case of the terrapin the results were very striking and easily obtained. The data from one of the experiments are as follows:

Locke's solution saturated with	Rate of outflow per minute
O ₂ 95 per cent, N 5 per cent	21.5 c.c.
O ₂ 95 per cent, CO ₂ 5 per cent	33.0 c.c.
O ₂ 95 per cent, N 5 per cent	31.5 c.c.
O ₂ 95 per cent, CO ₂ 5 per cent	38.25 c.c.

The perfusion pressure was held at 30 centimetres of water, and the change of gas-saturated solution was accomplished by a two-way stop-

TABLE III.
DATA FROM PERFUSION OF RATS' LUNGS.

Experiment.	Perfusion fluid saturated with	Rate of outflow per minute.
	per cent.	drops.
I	O ₂ 90, N 10	42
	O ₂ 90, CO ₂ 10	45
	O ₂ 90, N 10	26
	O ₂ 90, CO ₂ 10	23
	O ₂ 90, N 10	23
	O ₂ 90, CO ₂ 10	30
II	O ₂ 75 N 25	60
	O ₂ 75, CO ₂ 25	67
	65
	O ₂ 75, N 25	56
	O ₂ 75, CO ₂ 25	68
III	O ₂ 75, CO ₂ 25	15
	O ₂ 75, N 25	9
	O ₂ 75, CO ₂ 25	17

cock joining the two flasks. The outflow was recorded by a double dipping-bucket with electric contacts constructed on the principle of the U. S. Weather Bureau rain gauge. Each dip of the bucket registered 3 c.c. of outflow. Five minutes elapsed between each determination.

The same arrangement and head of pressure was used in perfusing the rats' lungs. In addition the lungs were placed in an artificial thorax pictured in Fig. 2. Cannulas were inserted in the trachea, pulmonary artery, and aorta. After the contained blood was washed out the cannulas were passed through the stopper (A), and the lungs were transferred to the chamber. Negative pressure was produced and maintained constant by the suction bottle (B). This bottle also aspirated the fluid which escaped through the walls of the lungs, from which it was led to the outflow recorder. During the per-

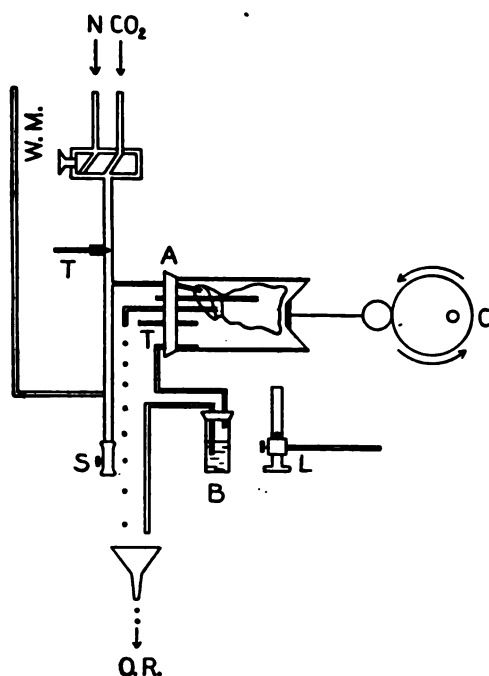


FIGURE 2. — A, stopper; B, suction bottle; T, thermometer; S, screw clamp; L, lamp; C, revolving cam; W. M., water manometer; O. R., outflow recorder.

fusion the cam (C) rhythmically altered the pressure within the chamber. The temperature was registered by the thermometer (T) and maintained constant by adjusting the screw clamp (S), which altered the rate of escape of fluid from the pressure flask in use.

With this apparatus we performed three conclusive experiments, the record of one of which is reproduced in Fig. 3. The data from these experiments are given in Table III.

The figures here given show that carbon dioxide acts to relax the

tone of the pulmonary vascular bed. In the terrapin 5 per cent is sufficient to give definite results. In the rat, the technique and maintenance of normal conditions being much more difficult, higher percentages are necessary to bring out the effect. Variations in temperature disturb the results. Particular care was observed in regard to this point in the second and third experiments. They are, in consequence, of more definite value.

DISCUSSION.

The results presented in the foregoing paragraphs indicate that the increased output of the mammalian heart under the influence of carbon dioxide, as observed by Jerusalem and Starling, may be due indirectly to the effect upon the pulmonary vascular bed.

The increase in rate of flow through the pulmonary vessels as observed by us in the terrapin and rat corresponds roughly with the increase in cardiac output as observed by Jerusalem and Starling in the lung-heart preparation.

Finally, Jerusalem and Starling have suggested that the rise in general arterial pressure consequent to the inhalation of increased amounts of carbon dioxide may be referred to an increased cardiac efficiency. This idea is not supported by the results of Kaya and Starling⁶ on the spinal animal, nor is it in accordance with our own results. The rise of arterial pressure in the intact animal on breathing carbon dioxide is probably a central rather than a peripheral effect.

Although we believe our results show that carbon dioxide is detrimental to the efficiency of both cold and warm blooded hearts when isolated from the body, it is proper to state that the evidence presented gives no information as to the effect, beneficial or otherwise, of this gas upon the body as a whole.

CONCLUSIONS.

1. Carbon dioxide, 2½ per cent in an excess of oxygen, when dissolved in the perfusion fluid, depresses the activity of the isolated terrapin's heart.

⁶ KAYA and STARLING: *Journal of physiology*, 1909, **xxxix**, p. 346.

2. Carbon dioxide, 3 per cent, likewise despresses the activity of the isolated cat's heart.

3. Carbon dioxide in an excess of oxygen dissolved in the fluid perfusing the lungs of terrapins and rats materially increases the rate of outflow.

4. This last result is suggested as an explanation to account for the difference in the results here presented and those of Jerusalem and Starling.

ON THE SECRETION OF URINE IN BIRDS.

By N. C. SHARPE.

[*From the Laboratory of Pharmacology of the University of Toronto.*]

AS the urine of birds is relatively rich in urates, it appeared possible that some of the questions connected with their secretion might be solved by employing these animals. Before, however, these inquiries could be undertaken, some knowledge of the character of their urine had to be obtained and also of the effects of diuretic substances upon both its amount and composition. At the suggestion of Prof. V. E. Henderson, I gladly undertook this preliminary investigation.

Hens were used in all the experiments. They were fed while in the laboratory with oats and water only. When kept in cages from which all excreta could be collected, practically no urine was found in the collecting vessel (in one case 3 c.c. in twenty-four hours). Urine obtained from cannulas inserted into the ureters was usually abundant. It was in most cases clear, but in some contained a heavy white deposit consisting of urates and in other cases some mucus. These facts point to a rapid absorption of fluid from the rectum, and in one experiment in which the bird was not in the best of condition 20 c.c. of water were absorbed in the course of one hour by a tube of rectum and cloaca barely 10 cm. in length and weighing about 5 gm. That such an absorption of water occurs was deduced by Wiener¹ from his experiments on hens with an artificial anus and from which urine and fæces could be collected separately. He noted that the urine was abundant and that the hens drank abnormally large amounts of water.

The hens were in all cases anæsthetized with urethane, given by rectal injection, 1.5 gm. per kilo, followed by ether during the time of operation and in most of the experiments throughout its course; in others subsequent additional doses of urethane were given. Artificial

¹ WIENER: Beiträge zur chemischen Physiologie und Pathologie, 1902, ii, p. 42.

respiration was used in almost every case, and the ether was given mechanically by the pump. Great care had to be taken to keep the anaesthesia uniform, as variations in its depth were almost invariably followed by changes in the rate of secretion. Very considerable differences in the amount of ether that had to be administered to different fowls were noted. The respiratory centre seemed especially liable to depression if the amount of ether slightly surpassed that necessary for complete anaesthesia.

The blood pressure was obtained by means of a mercury manometer connected to one of the common carotid arteries by a Harvard metal cannula which was coated with paraffin. In the early stages of any experiment much trouble was caused by rings of constriction which frequently developed in the arteries as the result of the necessary handling. Injections were made by means of a tapped cannula inserted into one of the external jugular veins. Metal cannulas were inserted into the ureters just above their entrance into the cloaca. In some of the early experiments the urine from the left side was obtained by tying a cannula into the anus and ligating the rectum above the cloaca. The urine flow was recorded by drop counters of the Loewi type, and the urine was subsequently collected into graduates for analysis. The ureter cannulas had to be carefully watched, as there was a danger of their becoming plugged when the urine contained much mucus or a heavy deposit. The operative procedures, including the insertion of the ureter cannulas, did not seem to inhibit the flow of urine; in many cases the cannulas filled almost at once. In the few cases where the absorptive powers of the rectum were tested, it was ligated just below the entrance of the two blind pouches, about 5 cm. from the anus; a pipette was then tied into the anus from which fluid under but slight pressure could be introduced. During the experiment the fowls were kept warm by means of an electrically heated box. Changes in surface temperature had a marked effect on the flow.

The total nitrogen was estimated by Kjeldahl's method; the uric acid by precipitation with ammonium sulphate and ammonia and titration with permanganate; the ammonia by neutralized formalin and sodium hydrate; the chlorides by Volhard's silver nitrate and sulphocyanide method; the phosphates by titration with uranium acetate. For these determinations considerable quantities of urine were needed, and in most cases sufficient could not be obtained during the effect

of a single injection of a diuretic, but repeated injections had to be resorted to.

The hens varied in weight from 1.1 to 2.7 K. No correlation between the weight and the quantity of urine was noted. The quantities of urine collected and the times taken during their collection under normal conditions are shown in Table I. The amounts of the various constituents are given in grams per 100 c.c. These same results re-calculated for twenty-four-hour periods are shown in Table I.

TABLE I.¹

Experiment no.	Weight of hen.	Time in minutes.	Quantity in c.c.	Total N in mgm.	Uric acid in mgm.	Uric acid calc. as N.	Ammonia.	Chlorides.	Phosphates.
15	1.36	80	31.0	196.7	82.7	29.3	...	765.6	...
20	1.80	60	26.4	182.7	116.4	41.3	11.5	248.4	...
23	2.10	90	41.3	159.6	153.7	54.5	...	280.0	...
25	1.50	90	31.7	217.7	207.5	...
32	2.70	12	6.3	99.7	35.3
35	2.00	75	56.2	220.1	98.6	34.9	100.0
36	1.36	22	13.9	186.6
37	1.50	120	58.0	238.0	132.0	46.8	10.2	352.8	80.0
41	1.10	30	14.2	180.6	204.5	72.5	17.0	110.0
Mean	1.90	198.8	143.6	50.9	13.6	486.5	95.0
¹ Highest and lowest quantities in heavy type. Quantities in mgm.									

As will be seen from Table II, the quantity of urine varied from 500 to 1000 c.c. Paton² obtained from hens with an artificial anus 600-1000 c.c. The urine was usually clear pale amber in color and either neutral or alkaline to litmus, acid to phenolphthalein. In those cases where it was cloudy the deposit seemed to be due to urates.

² PATON: *Journal of physiology*, 1909, xxxix, p. 485.

Urine-containing deposits were diluted and warmed before being used for analysis. In no case was albumen present.

The values shown in Table II for total nitrogen and uric acid may be compared with the following facts noted incidentally by other observers. Kionka,³ in his paper on "Bird-gout," gives the following

TABLE II.
ABOVE RE-CALCULATED FOR TWENTY-FOUR-HOUR PERIODS.¹

Experiment no.	Quantity of urine in c.c.	Total N in gm.	Uric acid in gm.	Ammonia in gm.	Chlorides in gm.	Phosphates.
15	558	1.097	0.46	4.27
20	634	1.16	0.74	0.073	1.70
23	660	1.05	1.01	1.84
25	507	1.10	1.05
32	756	0.75
35	1000	2.20	0.98	1.0
36	910	1.69
37	696	1.66	0.92	0.07	2.45	0.55
41	680	1.23	1.40	0.11	0.75
Mean	750	1.63	0.93	0.93	2.66	0.77

¹ Highest and lowest in heavy type.

analysis of urine and fæces: solids 6.0–10.5 gm., water 35–62 gm., nitrogen 0.48–0.82 gm., uric acid 1.0–1.75 gm., ammonia 0.09–0.12 gm. Von Schröder⁴ found the uric acid in hens to vary from 1.35 to 1.56 gm. Von Mach⁵ in fæces, nitrogen 0.60–0.85, uric acid

³ KIONKA: Archiv für experimentelle Pathologie and Pharmakologie, 1900, xliii, p. 186.

⁴ VON SCHRÖDER: Zeitschrift für physiologische Chemie, 1878, ii, p. 228.

⁵ VON MACH: Archiv für experimentelle Pathologie und Pharmakologie, 1887, xxiv, p. 389.

0.77-1.25. Wiener¹ in fæces found the uric acid to vary from 1.41 to 1.68 gm.

The relation of the rate of flow to the blood pressure varied greatly. In many cases with as low a pressure as 70 mm. of mercury there was a good flow, and in other cases, even with a pressure as high as 130, there would be no flow for hours. In other cases with a low pressure

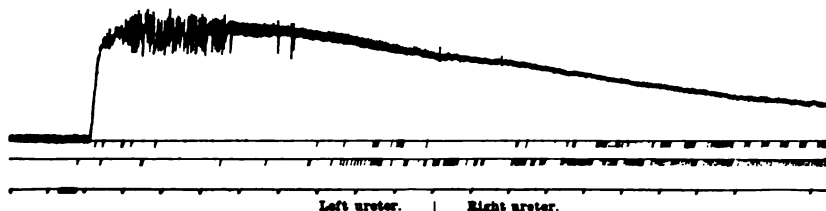


FIGURE 1. — One third the original size. The first or uppermost curve gives the carotid blood pressure recorded by a mercury manometer; the second, the drops flowing from the left, and the third, the flow from the right ureter. The lowest line records the time in half minutes, beginning at 12.28, as well as the atmospheric pressure. In successive intervals of two minutes, there flowed from the left ureter 6, 12, 38, and 44 drops, and from the right ureter 4, 8, 40, 65, and 66 drops.

there would be no flow till the blood pressure was raised by some change in anæsthesia or adrenalin was administered, when a prompt flow would be the result. In Experiment 7, after the bird had been several hours on the table and prolonged diuresis increased by the administration of diuretics had taken place, the blood pressure was very low and the urine flow ceased almost completely; an injection of 20 c.c. of normal saline increased the flow slightly, but subsequent to the rise in pressure brought about by an injection of adrenalin, marked flow began (see Fig. 1).

Diuretics. — The effect of injections of 1 c.c. of a 1 per cent solution of caffein sodium benzoate are shown in Tables III and IV and in Fig. 2. The response was usually very prompt, as shown in the curve which is taken from a typical experiment. The blood pressure was usually little affected. The greatest change noted was in Experiment 23, where there was a rise of 12 mm.; the previous level was reached long before diuresis was complete. The increased flow, greatest at first, often lasted for a considerable time (fifteen to twenty minutes), though at a gradually decreasing rate. Second injections usually produced less effect than the first, but even third injections usually produced some effect. No depressing effect of second injections, such

TABLE III.

THE EFFECT OF INJECTION OF 1 C.C. 1 PER CENT SOL. CAFFEIN SODIUM BENZOATE.

Exper. no.	Injection.	Previous rate.	Subsequent rate.	Duration till normal rate reached again.
20	{ First	1.8 c.c. in 10 min.	2.6 c.c. in 10 min.	15 min.
	{ Second	3 drops in 2 min.	9 drops in 2 min.	4 min.
23	{ First	0.6 c.c. in 5 min.	2 c.c. in 5 min.	25 min.
	{ Second	0.6 c.c. in 5 min.	0.9 c.c. in 5 min.	20 min.
	{ Third	3 drops in 2 min.	4 drops in 2 min.	...
	{ Fourth	2 drops in 2 min.	2 drops in 2 min.	...
32	{ First	31 drops in 2 min.	49-125 drops in 2 min.	36 min.
	{ Second	14 drops in 2 min.	22-31 drops in 2 min.	40 min.
27	{ First	18.5 c.c. in 21 min.	24 c.c. in 21 min.	...
	{ Second	10 c.c. in 21 min.	14 c.c. in 21 min.	...
	{ Third	11.7 c.c. in 21 min.	13.3 c.c. in 21 min.	...
31	{ First	No drop in 2 min.	62 drops in 2 min.	...
	{ Second	19 drops in 2 min.	34 drops in 2 min.	15 min.

TABLE IV.

EFFECT OF THE INJECTION OF CAFFEIN ON THE URINARY CONSTITUENTS.

Exper. no.		Rate c.c. in min.	Total N in mgm.	Uric acid in mgm.	Total N per 100 c.c. in mgm.	Uric acid per 100 c.c. in mgm.
23	{ Normal	9.1 in 60	14.5	13.98	159.6	153.7
	{ Caffein	11.5 in 60	24.25	11.84	210.9	103.0
32	{ Normal	18.5 in 21	17.38	9.45	93.9	41.1
	{ Caffein	24.0 in 21	24.20	11.15	110.9	46.9
27	{ Normal	42.0 in 80	84.00	41.89	200.0	99.75
	{ Caffein	74.0 in 80	142.30	42.47	192.3	57.40¾

as was observed by Barcroft and Straub⁶ in cats was noted. In equal quantities of urine caffeine usually increased the total nitrogen and decreased the uric acid. When the secretion of equal times are compared, as in the third and fourth columns of Table IV, but little change in the output of uric acid can be seen.

Injections of 2 c.c. of a 4 per cent solution of sodium sulphate gave a prompt diuresis, but usually one less marked than by the above dose of caffeine, and the diuretic period was usually short. Second and even third injections usually produced some effect. The blood pressure was little affected by the injections, the greatest increase observed being 6 mm. In Experiment 41, which seemed typical of the effect upon the uric acid and total nitrogen excretions, the analyses showed, normal total N 180.6 mgm. per 100 c.c., uric acid 204.5 mgm.; after sodium sulphate 219.6 mgm. N and 225.0 mgm. uric acid.

But few experiments were made with potassium nitrate. In Experiment 39 the previous rate of 3-6 drops in two minutes was increased to 12-20, and the increase continued for twenty minutes; the blood pressure was unaffected.

In but one or two experiments injections of 2 c.c. of a 10 per cent solution of glucose were given, resulting in an increase in flow; *e. g.*, in Experiment 31 the rate increased from 4-6 drops per two minutes to 15 drops.

The injection of piperazin 100 mgm. in 1 c.c. of distilled water produced in practically every instance a prompt response. The flow was usually well sustained, and often seemed to come to a rather abrupt termination some twenty minutes after the injection had been given.

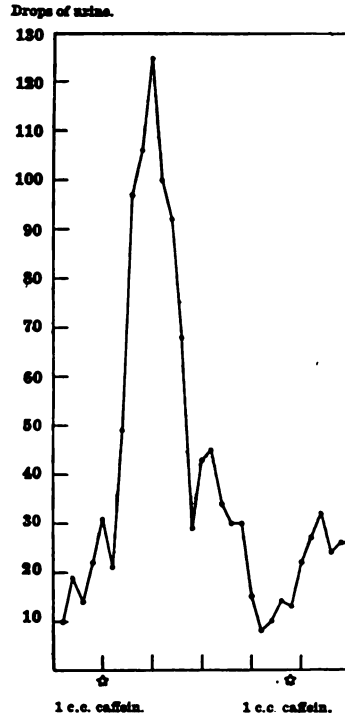


FIGURE 2.—From Exp. 32. The ordinates indicate drops of urine and the abscissal time in two-minute periods. At points marked with a star 1 c.c. of caffeine was given.

⁶ BARCROFT and STRAUB: Journal of physiology, 1910, xli, p. 145.

TABLE V.
EFFECT OF SODIUM SULPHATE ON THE RATE OF URINE FLOW.

Exper. no.	Injection.	Previous rate.	Subsequent rate.	Duration till the normal rate was reached again.
23	First	0.4 c.c. in 4 min.	0.9 c.c. in 4 min.	20 min.
	Second	2 drops in 2 min.	4 drops in 2 min.	8 min.
27	First	11 drops in 2 min.	18 drops in 2 min.	20 min.
	Second	10 drops in 2 min.	17 drops in 2 min.	20 min.
30	First	9 drops in 2 min.	15 drops in 2 min.	15 min.
	Second	8 drops in 2 min.	9 drops in 2 min.
33	First	13 drops in 2 min.	24 drops in 2 min.	15 min.
	Second	20 drops in 2 min.	32 drops in 2 min.	15 min.
	Third	14 drops in 2 min.	21 drops in 2 min.	10 min.

TABLE VI.
THE EFFECT OF PIPERAZIN 100 MGM. ON THE RATE OF URINE FLOW.

Exper. no.	Injection.	Previous rate.	Subsequent rate.	Duration till normal rate was reached again.
36	First	23 drops in 2 min.	43-60 drops in 2 min.	...
37	First	4 drops in 2 min.	11 drops in 2 min.	...
34	First	11 drops in 2 min.	13-29 drops in 2 min.	25 min.
	Second	12 drops in 2 min.	19 drops in 2 min.	...
38	First	24 drops in 5 min.	48 drops in 2 min.	...
	Second	27 drops in 5 min.	38 drops in 2 min.	...

The effect on the blood pressure was never marked, in some cases a slight rise, in others a slight fall; neither change seemed to influence the flow in any degree. Table VI shows the effect on the rate of secre-

tion. In Experiment 37, which seemed to be one of the most typical, the normal N was 238.0 mgm. per 100 c.c. after piperazine 270.0, the uric acid before 132.0 and afterwards 202.9. All our experiments seemed to point to piperazine having some more or less marked influ-

Drops of urine.

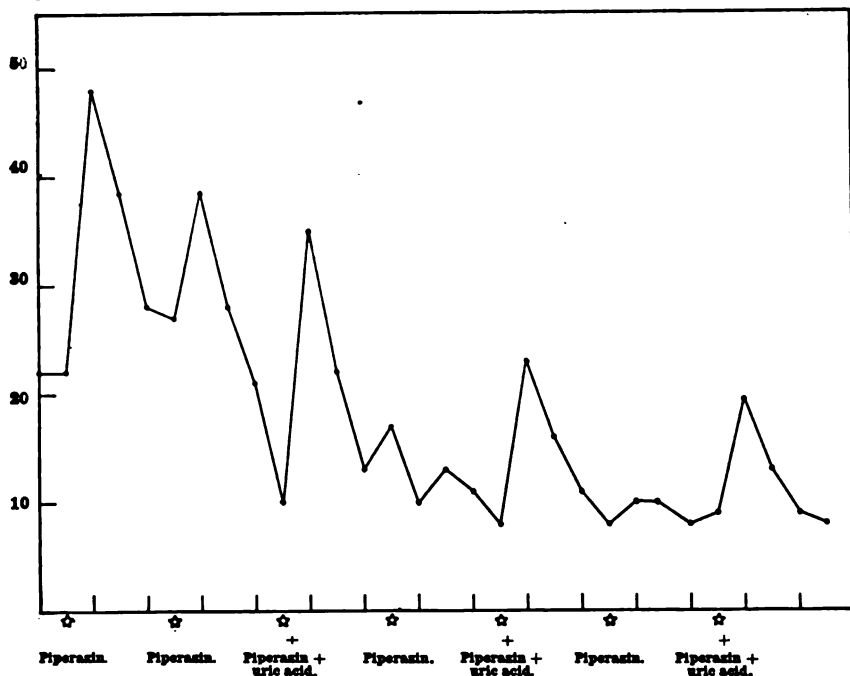


FIGURE 3. — The ordinates indicate drops of urine and the abscissal time in five-minute periods. At points marked with a star piperazin was given; where the cross is added uric acid was also given.

ence on the excretion of uric acid. As it seemed possible that any direct diuretic effect of the piperazin might be augmented by the increased output of uric acid that accompanied it, the following experiment was tried, the results of which are shown graphically in Fig. 3. As will be seen, two successive piperazin injections each produced a marked effect; though the second, beginning from a lower normal, produced a much less total secretion during the same time period, gradual decline in the response due to successive injections seemed probable. The third injection consisted of the same amount of piperazin, but held 200 mgm. of uric acid in solution; it will be noted

that the decline in total production is by no means as great as between the first and second injections. The fourth injection consisted of piperazin alone and was strikingly inefficient. The fifth injection containing uric acid was more efficient than the fourth, and the seventh which contained it than the sixth that did not. The total urine production during this period of repeated injection was 39 c.c., which contained 261 mgm. of uric acid; the same amount of normal urine would have contained only 135 mgm.

CONCLUSIONS.

The urine of hens is usually abundant and clear as it leaves the ureters, and its water content must be largely reabsorbed from the bowel.

The ordinary diuretics produce increases in urine flow similar to that found in other animals.

THE RELATION OF POTASSIUM SALTS AND OTHER SUBSTANCES TO LOCAL ANÆSTHESIA OF NERVES.

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INTRODUCTION.

THAT there exists a striking parallel between the degree of anæsthesia produced by the narcotic drugs which are fat solvents and the physico-chemical affinity between each of these and the lipoids has been demonstrated independently by Overton¹ and Meyer.² According to these authors a drug is active as a narcotic in proportion as its solution tension is high for lipoids and low for aqueous compounds.

The lipoids, and especially those of the phosphatid group, are always characterized by a varying percentage of inorganic salts, of which sodium and potassium predominate. The presence of the former has been noted by Thudicum,³ and by Fränkel,⁴ and recently Koch and Pike⁵ have analyzed various samples of kephalin and lecithin and find that both sodium and potassium occur in these compounds in considerable amounts, and that in the kephalin the potassium is greatly in excess of the sodium. They observed that the potassium was present in some combination other than the chlorides, phosphates, or sulphates and suggested the existence of an ion-colloid combination.

The investigation outlined in the following pages deals with changes which occur in the potassium content of the lipoids of the nerve fibre after exposure to anæsthetic vapors.

¹ OVERTON: Studien über Narkose, Jena, 1901.

² MEYER: Archiv für experimentelle Pathologie und Pharmakologie, 1889, xlii, pp. 109, 119, and 1909, xlii, p. 338.

³ THUDICUM: Die chemische Konstitution des Gehirns des Menschen und der Tiere, Tübingen, 1901.

⁴ FRÄNKEL: Wiener medicinische Wochenschrift, 1909, p. 2741.

⁵ KOCH and PIKE: Journal of pharmacology and experimental therapeutics, 1910, ii, p. 245.

METHOD.

An exceedingly sensitive reagent to show minute traces of potassium was introduced into micro-chemistry by Macallum.⁶ This compound, the hexanitrite of cobalt and sodium, $\text{CoNa}_3(\text{NO}_2)_6$, gives with a minute quantity of potassium, either free or in combination, an orange yellow precipitate, the triple nitrite of cobalt, sodium, and potassium. This yellow compound is rendered more evident by adding ammonium sulphide after the tissue has been washed free from all the remaining uncombined reagent.

The localization in normal nerve fibres and in nerve cells has been thoroughly studied by Macallum, who found that in medullated nerves potassium was never present in the substance of the axis cylinder, but occurred at the nodes of Ranvier, in the neurokeratin, in a thin layer on the inner surface of the medullary sheath, and also irregularly distributed through the latter structure. In non-medullated nerves a faint potassium reaction occurred in the neurilemmas, but was absent from the axon, and in the nerve cells no trace of this element was ever found.

The reagent used in all of the micro-chemical experiments was prepared according to the method of Macallum, and the technique employed was similar to that recommended by this investigator.

One precaution, however, was found necessary. Since the application of any mechanical injury such as cutting, tearing, pressure, and heating previous to immersion of the nerve in the reagent produces an increase of the potassium similar to that observed in anæsthetized nerves, extreme care must be taken in handling the nerve preparations. This observation concerning the occurrence of this salt after injury agrees with that of MacDonald,⁷ who was able to produce at any point arbitrarily selected in the nerve fibre a deposit of potassium salt on injury.

It was frequently noted in experiments on dogs' nerves that when these were cut, a cessation of conduction immediately followed for a short distance from the site of injury. In the majority of cases this was not permanent, the power of conduction being restored in the injured part, usually in a few minutes. The extent of this area corre-

⁶ MACALLUM: Proceedings of the Royal Society, 1898, B., lxiii, p. 467.

⁷ MACDONALD: Proceedings of the Royal Society, B., 1906, lxxvi, p. 332.

sponded relatively to the intensity of the injury, being least marked if cut with a very sharp scalpel, and considerable if a blunt instrument were used. These cut ends always showed an abundant deposit of potassium which was most marked at the immediate point of injury, with a gradual diminution extending outward on either side from this. It was noted, however, that the area of the nerve in which after injury there was no response to stimulation extended over a greater stretch than that in which the potassium was revealed by means of the reagent. As a result of many experiments it was apparent that the amount of potassium which could be thus demonstrated after injury depended, up to a point at which the maximum was reached, upon the extent of the injury, and that under proper conditions it could be indeed reduced to a minimum. In a few instances, where the sciatic nerve of a frog was treated with the reagent *in situ*, if sufficient care had been taken in dissecting back the muscles, there was in short stretches of the nerve a complete absence of any coloration except in the blood vessels supplying the nerve. As the erythrocytes in the vessels lying in the deeper parts of the nerve frequently gave a yellow reaction with the cobalt, the lack of potassium was apparently not due to an insufficient penetration of the reagent into the nerve fibre.

The peripheral nerves, because of the large amount of lipoids contained in their medullary sheaths and because of the sharp demarcation between the lipoids in those structures and the proteid substances of the axon, were selected as being best adapted for studies of the potassium distribution in anæsthesia.

Various nerves from dogs, cats, rabbits, guinea pigs, and frogs were used, but as the sciatic nerves could be removed and handled with the least injury in the last-mentioned animals, these gave the best microchemical preparations, and in the latter experiments were used almost exclusively.

The sciatic nerves from either side of the pithed frog were carefully removed, as in a nerve muscle preparation, and were then mounted in two moist chambers, one of which was kept moist with Ringer's solution, and the atmosphere of the other was saturated with the vapors of the anæsthetic used.

The irritability of both nerves was tested by an induction current sufficient to give a contraction of the gastrocnemius muscle, and at intervals during an experiment the loss of conductivity was noted in either nerve by testing with the same current.

At the end of definite periods of time both nerves were similarly treated with the potassium reagent, subsequently washed thoroughly with distilled water and mounted in 50 per cent glycerine.

THE POTASSIUM CONTENT OF ANÆSTHETIZED NERVES.

The anæsthetized nerve appeared a deep yellow, while the normal control nerve, except at points where injury might have occurred in handling before immersion in the reagent, remained colorless. A series showing a gradation from an almost imperceptible yellow to a deep orange was produced by varying the length of time of the exposure of the nerve to the anæsthetic vapors.

Similar results were obtained by varying the amounts of the anæsthetics used, with the three anæsthetics employed, *viz.*, chloroform, ether and ethyl alcohol. Upon exposure of the nerve to a saturated vapor, a maximum intensity of color was reached with chloroform in about fifteen minutes; with ether a longer period of time was required, and with alcohol the reaction developed still more slowly. A typical yellow coloration was obtained in any area selected by exposing only that part of the nerve fibre.

On microscopic examination of the anæsthetized nerve, the greater amount of the potassium appeared as large orange-yellow crystalline masses irregularly distributed over the surface of the nerve. In the teased preparation the precipitate was constantly found at the nodes and also throughout the medullary sheath, but with no definite localization in this latter structure. In some of the fibrils the whole outer surface was dotted with minute yellow particles, while in others here and there, on the inner as well as on the outer surface of the medullary sheath, masses of the most varying size and contour were found variously distributed. The axon, except at the nodes, was comparatively free from any deposits of the potassium salt, although small irregular masses were occasionally met with in its outer layer.

POTASSIUM SALTS BLOCK THE NERVE IMPULSE.

Having established that concurrently with anæsthesia in the nerve fibre there was an increase in the potassium as revealed by the Macalium reagent, a series of experiments was next undertaken to test whether

potassium salts injected into the nerve would possess any inhibitory action on the nerve impulse. For this purpose the sciatics and the nerves from the brachial plexus supplying the fore-paw in dogs were used. The dogs were anæsthetized with ether, and the nerves carefully exposed and cut as close to the cord as possible. Immediately previous to an injection of the solutions used the nerves were stimulated with the electrodes from an induction coil, and directly following the injection of 2 to 5 minims of the solution of the various salts, they were again tested with a current of the same strength. Stimulation was then repeated at short intervals until conduction was restored in the nerve and the total duration of the inhibition noted. The salts of potassium used were as follows: chloride, iodide, bromide, phosphate, bicarbonate, disulphate, nitrate, chlorate, fluoride, formate, citrate, acetate, oxalate, lactate, benzoate, ferrocyanide, cyanide, tartrate, and sodium potassium tartrate. All of these except the lactate and hydrate were used in a 2 per cent solution, and subsequently in concentration isotonic with 0.64 per cent sodium chloride (as the chlorides in the serum of dog's blood are slightly higher than 0.64 per cent, the concentrations used were somewhat lower than an isotonic one). The lactate and hydrate were used in the concentration noted in the appended table.

With the exception of the oxalate, citrate, acetate, tartrate and sodium potassium tartrate, all the salts blocked the conduction from one to three hours. Usually the block was instantaneous but in some cases it was delayed three minutes. These results are paralleled by those given by Matthews,⁸ who obtained a rapid loss of irritability in the sciatic nerves of frogs by immersion in various salts of potassium.

The duration of the anæsthesia depended upon the amount injected, upon the size of the nerve, and the depth into the interior of the nerve that the hypodermic needle penetrated. The larger the quantity injected and the smaller the nerve the more quickly did the block occur and the more permanent was the inhibition. With the sodium potassium tartrate and with the citrate, oxalate, acetate, and tartrate of potassium an inhibitory effect on nerve conduction was seldom apparent if a small quantity, such as from 2 to 5 minims of the solutions, was used. However, an injection of 15 to 20 minims,

⁸ MATTHEWS: *this Journal*, 1904, xi, p. 455.

TABLE I.
POTASSIUM SALTS.

Solution 0.64 per cent.	Amount in minims injected.	Length of time in minutes after injection before effective.	Average duration of suspension of conduction.	
			h.	min.
Chloride	2	Instantaneous	1	20
Iodide	2	Instantaneous	1	20
Bromide	2	Instantaneous	1	43
Phosphate	2	Instantaneous	1	13
Carbonate	3	1	1	48
Disulphate	3	Instantaneous	1	00
Nitrate	2	Instantaneous	1	27
Chlorate	3	1½	2	43
Fluoride	3	Instantaneous	2	21
Ferrocyanide	3	Instantaneous	4	36 ¹
Ferricyanide	2	Instantaneous	2	30
Cyanide	2	Instantaneous	1	00
Formate	2	1	1	38
Citrate	2	No inhibition	.	..
Acetate	4	No inhibition	.	..
Oxalate	3	No inhibition	.	..
Lactate	4	Instantaneous	1	48
Benzoate	3	1	1	02
Tartrate	5	No inhibition	.	..
Sodium potassium tartrate	5	No inhibition	.	..
.....
¹ Block effective at time of death.				

and especially if this was rapidly injected so as to cause considerable distention of the nerve, produced a block in conduction varying from fifteen to forty-five minutes and was apparently due for the greater

TABLE I (continued).

POTASSIUM SALTS.

2 per cent solutions.	Amount injected in minims.	Length of time in minutes after injection before effective.	Average duration of suspension of conduction.
Chloride	2	Instantaneous	h. min. 2 21
Bromide	2	Instantaneous	1 40
Iodide	2	Instantaneous	1 30
Carbonate	2	Instantaneous	1 45
Nitrate	3	Instantaneous	1 25
Chlorate	3	$\frac{1}{2}$	1 30
Fluoride	3	Instantaneous	2 00
Ferrocyanide	3	1	3 50
Ferricyanide	3	1	4 00
Cyanide	2	Instantaneous	1 54
Formate	3	Instantaneous	1 05
Citrate	4	2	0 15
Acetate	5	No inhibition	. ..
Oxalate	3	No inhibition	. ..
Lactate	5	Instantaneous	1 48
Tartrate	5	No inhibition	. ..
Sodium potassium tartrate .	5	No inhibition	. ..
Hydrate 2 per cent	2	Instantaneous	5 45 ¹
Hydrate 0.5 per cent	2	Instantaneous	5 30 ¹
Hydrate 0.25 per cent . . .	2	Instantaneous	25
Hydrate 0.6 per cent. . . .	2	Instantaneous	12
¹ No response to stimulation at time of death.			

part to mechanical effects. Injection of a 2 per cent solution of potassium hydrate produced a block which was effective up to the time of the dog's death, from five to six hours later. Microscopic examination of

the injected area of these nerves revealed at this point a widespread disintegration of the medullary sheaths. This action upon the lipid substance was less marked as the concentration of the hydrate was decreased.

Sensory nerves, as demonstrated by injections into the vagus and superior laryngeal, gave anæsthetic effects analogous to those obtained in the motor nerves. By injection into the vagus of potassium salts in the concentration above mentioned the author obtained results similar to those reported by Dixon⁹ on the application of cocaine, and by Meltzer and Auer¹⁰ upon immersion of the nerve fibre in solutions of magnesium sulphate and chloride.

EFFECT OF OTHER SUBSTANCES ON NERVE CONDITION.

The sodium salts, *viz.*, chloride, bromide, iodide, chlorate, phosphate, fluoride, nitrite, bromate, carbonate, nitrate, chromate, acetate, tartrate, oxalate, citrate, disulphate, sulphite, succinate, and sulphate in solutions, isotonic to 0.64 per cent and 0.9 per cent on injection, gave no anæsthetic effects. Occasionally, if a very considerable quantity were injected, a block, resulting apparently from injury due to pressure, was obtained. This was also produced by such substances as distilled water, egg albumin, olive oil, and thin gelatin, and lasted usually for five to seven minutes, but occasionally extended to fifteen minutes. When potassium salts were added to the egg white and gelatin, a prolonged block could be obtained.

Besides the sodium salts, the chloride, bromide, and citrate of lithium in solutions isotonic to 0.64 per cent sodium chloride on injection gave no appreciable diminution in the conducting power of the nerve. Magnesium sulphate, chloride, acetate, bromide, and nitrate likewise gave similar results in solutions isotonic to 0.64 per cent sodium chloride. However, using solutions approximately equimolecular to 0.9 per cent sodium chloride, *i. e.*, magnesium chloride 3.1 per cent and magnesium sulphate 3.7 per cent, concentrations with which Meltzer and Auer¹¹ obtained a block in rabbits' sciatic nerves when these solutions were applied locally to the nerve fibres,

⁹ DIXON: *Journal of physiology*, 1905, xxxii, p. 87.

¹⁰ MELTZER and AUER: *this Journal*, 1906, xvi, p. 233.

¹¹ MELTZER and AUER: *Loc. cit.*

a definite suspension of conductivity resulted, but this was of short duration. Increasing the concentrations gave a correspondingly prolonged inhibition of conduction. Barium bromide, chloride, iodide, and nitrate likewise gave negative results when used in similar isotonic concentrations.

Among the salts of the heavy metals, isotonic silver nitrate, lead acetate and lead nitrate in saturated solutions, 1 per cent gold chloride and 1 per cent platinum chloride gave instantaneous, and in the first three mentioned cases permanent blocks.

With the silver nitrate microscopic examination of the fibre after exposure to sunlight revealed at the nodes of Ranvier a blackened ring-like precipitate of the reduced chlorides and in many instances the typical crosses. Saturated picric and tannic acid produced anæsthesia lasting for about an hour.

SUMMARY.

1. In the medullated nerve fibres upon mechanical injury, upon heating and in anæsthesia produced by the lipid-solvent anæsthetics, there is an increase of potassium as demonstrated by the Macallum reagent of hexanitrite of sodium and cobalt.

2. Solutions, approximately isotonic to blood plasma, of the various potassium salts, with the exception of the tartrates, oxalate, citrate, and acetate, suspend conductivity when injected into the nerve fibre. Isotonic solutions of sodium, lithium, barium, and magnesium do not give this anæsthetic effect.

In conclusion I wish to express my thanks to Professor G. W. Crile for his invaluable advice; these investigations were carried on in his laboratory and were made possible by his generosity. I also gratefully acknowledge my indebtedness to Prof. A. B. Macallum of Toronto for advice as to technique, to Prof. G. N. Stewart for suggestions during the work, and to Dr. H. G. Sloan for assistance in a number of experiments.

ON FEEDING YOUNG WHITE RATS THE POSTERIOR AND THE ANTERIOR PARTS OF THE PITUITARY GLAND.¹

By T. B. ALDRICH.

IT has been assumed, and certain observations seem to warrant the assumption, that the pituitary gland, either through its own secretions or through the united influence of its secretions with some inter-related gland secretion, has an influence on the growth and nutrition of the body. Whether this growth is due to an increased or decreased activity of the gland has still to be decided. Marie² took the former view, while Wood-Hutchinson³ took the latter, even going so far as to attribute this influence (hormonic) to the anterior lobe alone.

The author, having been interested in the pituitary body for a number of years (especially in the pressor body contained in the posterior lobe), recently carried out some feeding experiments with the anterior lobe, employing young pups for this purpose.⁴ The results of these feeding experiments did not show any very marked influence on the growth of the pups collectively, as indicated by their increase in weight, although the growth of certain individuals appeared to be stimulated by the addition of this portion of the gland."

Professor Schäfer⁵ claims "that the addition of small amounts of pituitary substances [anterior] to the food of young animals appears to favor their growth; at least it does not impede or restrict it." On the other hand, Cushing⁶ states that repeated injections of the extracts of the anterior lobe alone, using the boiled extract or the emul-

¹ Read before the Eighth International Congress of Applied Chemistry, Washington and New York, September 4-13, 1912.

² MARIE: *Revue de médecine*, 1885-1886; also *Archives de médecine expérimentale*, 1891.

³ WOOD-HUTCHINSON: *Transactions of the Pan American Medical Congress*, 1894.

⁴ ALDRICH: *this Journal*, xxx, p. 320.

⁵ SCHAFFER: *Proceedings of the Royal Society*, 1909, lxxxi, p. 442.

⁶ CUSHING: *Journal of the American Medical Association*, 1909, lii, p. 255.

sion of fresh and aseptically removed glands, failed to produce any constant disturbances, except a definite loss of weight.

Carletti (1907) injected young animals (intraperitoneally) with a pituitary emulsion (presumably the whole gland), and if his results are to be accepted, they appear to indicate, if anything, a retardation in the growth of the bones, at any rate in the direction of length. Sandri fed young mice with pituitary (apparently with the crude gland to the exclusion of other food), and states that this caused an arrest of the growth, but there were no controls. He also injected young guinea-pigs with an emulsion of the gland, and found in these also the growth diminished.

From the citations given (which include all the observations extant, relative to this subject) it would seem that there is a great diversity of results, which might be attributed to the fact (especially the results of Carletti and Sandri) that the whole gland was employed instead of the separated parts, were it not for the observations of Cushing, who states specifically that he used the separated anterior lobe with the results that a definite loss of weight was observed. Since the writer's previous experiments are not in accord with any of the observations recorded, approaching, however, more closely Schäfer's results, it seemed desirable to repeat his work, and the experiments here recorded were carried out with the view of answering, if possible, the question as to whether either the posterior or anterior lobe of the pituitary gland has any marked influence on the growth of young animals as registered by their weight. In these experiments it seemed desirable to use young white rats instead of pups in order to conform to the conditions obtained by Schafer in his work.

EXPERIMENTAL.

I. **Feeding the posterior lobe.** — For this purpose ten healthy white rats, one month old, and all of the same litter, were employed. On February 13 these were separated into two groups of five each (two males and three females in each group), each rat being placed in a separate cage. All were fed the same diet for seven days, that is, until February 20, this consisting of bread and milk (1:2) mixed to rather a thick consistency, and a limited amount of whole corn.

On February 20 the experimental feeding was started, and continued for over three months, each rat being fed daily 30 gm. of the bread and milk mixture (prepared fresh every day), with which was intimately mixed either 50 mg. of desiccated, defatted posterior powder (group A) or the same amount of powdered ovary or testicle alternately (group B). The food was placed in screw-cap jars, with a hole in the cage barely large enough to allow the proper access of the head to the food, but not large enough to allow the food to be scattered around the cage by the feet and tail of the rat. The jars with food were weighed each day before being placed in the cages and again after twenty-four hours of feeding, the proper amount being deducted (this being determined previously from a number of weighings) for evaporation, the difference, with this deducted, being the amount consumed by the rats in twenty-four hours. The jars were thoroughly cleaned each day before refilling, and the cages scalded out every third day, filter paper being placed in the bottom of each. In addition to the feeding already described, each rat of both groups was allowed each day 5 kernels of whole corn. With the exceptions cited all the rats of both groups were treated in every respect the same. The results obtained are given below.

The table on page 97 gives the total weight of the two groups, A and B, together with the average weight of the rats in each group on February 13, when weaned, on February 17, and on February 20, when the addition of posterior lobe or ovary (desiccated) was made, and also their weights with few exceptions, every fourth day thereafter, for a period of over three months. The last column gives the excess of average weight of one group over the other.

The table on page 97 and Fig. 1 show that the weights of the two groups ran along nearly parallel. At no time during the three months did the weights vary by more than 13 gm., and the greater part of the time by only a few grams, 1-6. The average weight variation is still less, 0-3 gm.

The increase in average weight in per cent of original average weight is as follows:

	Posterior.	Controls.
First week (normal diet)	33 ⁷ / ₀	35 ⁷ / ₀
April 1	179 ⁷ / ₀	180 ⁷ / ₀
May 20 (whole period)	290 ⁷ / ₀	293 ⁷ / ₀

Feeding Young White Rats Parts of the Pituitary Gland. 97

POSTERIOR GROUP, A (5 rats, 2 males, and 3 females).			CONTROL GROUP, B (5 rats, 2 males, and 3 females).		
Date.	Total weight.	Average weight.	Total weight.	Average weight.	Excess av. wt. of one group over the other.
Feb. 13 ¹	gm. 212	gm. 42	gm. 211	gm. 42	0
17	243	49	251	50	B ² 1
20 ²	282	56	286	57	B 1
25	300	60	304	61	B 1
29	338	68	347	69	B 1
Mar. 4	381	76	389	78	B 2
8	421	84	423	85	B 1
12	454	91	462	92	B 1
16	485	97	492	98	B 1
20	512	102	511	102	0
24	530	106	530	106	0
28	575	115	573	115	0
Apr. 1	579	116	586	117	B 1
5	604	121	606	121	0
9	645	129	643	129	0
13	663	133	661	132	A ⁴ 1
17	686	137	683	137	0
21	706	141	700	140	A 1
25	721	144	717	143	A 1
29	741	148	745	149	B 1
May 3	758	152	767	153	B 1
7	764	153	770	154	B 1
11	784	157	795	159	B 2
15	791	158	804	161	B 3
20	819	164	826	165	B 1

¹ Start of normal feeding.
² Start of posterior feeding.

³ B = Control.
⁴ A = Posterior.

During the feeding the posterior group consumed 7932 gm. of milk and bread mixture (12.3 gm. posterior powder), the controls 7774 gm. (13 gm. desiccated ovary or testicle), or a difference of 158 gm. in favor of the former.

Fig. 1 shows rate of growth of the two groups of rats, posterior and control. Group A, plain

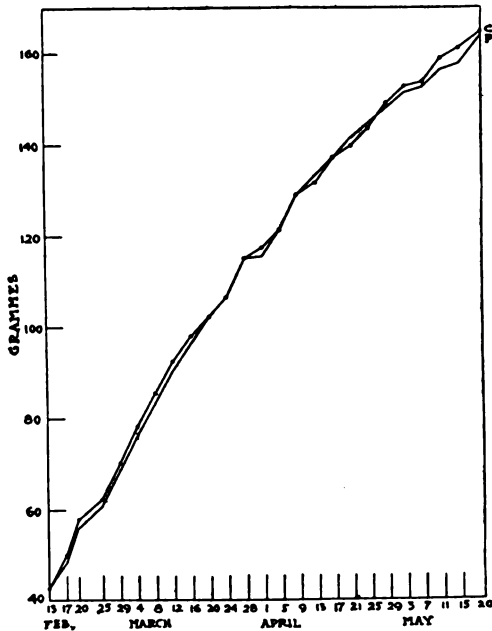


FIGURE 1. — From the table on page 97.

line, received the addition of posterior lobe of pituitary to food; group B, dotted line, the controls, received desiccated ovary and testicle. Addition was made February 20. The dates are marked upon the abscissa at proportionate intervals, while the weights are given as ordinates.

II. Feeding the anterior lobe.

— As in the feeding experiments with the posterior lobe, ten young healthy white rats, one month old, were employed.

On October 23 they were weighed and separated into two groups, each containing five rats (control, four males and one female; anterior fed, three males and two females), each rat being placed in a separate cage. All received the same food for seven days, that is, until October 30, when the experimental feeding was started. The experimental feeding continued for over three months.

The food, with the exception of the substitution of the desiccated non-defatted anterior lobe, was the same as in the previous experiment, and everything was carried out exactly the same. The amount of food consumed was also determined. The results obtained are given in the table on page 99 and in Fig. 2.

The table on page 99 gives the total weight of the two groups, A and B, together with the average weight of the rats in each group on

Feeding Young White Rats Parts of the Pituitary Gland. 99

ANTERIOR GROUP, A (5 rats, 3 males, and 2 females).			CONTROL GROUP, B (5 rats, 4 males, and 1 female).		
Date.	Total weight.	Average weight.	Total weight.	Average weight.	Excess av. wt. of one group over the other
Oct. 23-11	gm. 134	gm. 27	gm. 135	gm. 27	0
27	169	34	170	34	0
30 ¹	186	37	189	38	B ² 1
Nov. 4	207	41	222	44	B 3
8	241	48	263	53	B 5
12	275	55	302	60	B 5
16	294	59	325	65	B 6
20	337	67	370	74	B 7
24	344	69	380	76	B 7
28	366	73	416	83	B 10
Dec. 2	386	77	437	87	B 10
6	395	79	440	88	B 9
10	431	86	477	95	B 9
14	438	88	507	101	B 13
18	452	90	534	107	B 17
22	487	97	556	111	B 14
26	513	103	581	116	B 13
30	499	100	575	115	B 15
Jan. 3-12	532	106	602	120	B 14
8	565	113	650	130	B 17
12	580	116	665	133	B 17
16	600	120	690	138	B 18
20	638	128	734	147	B 19
24	638	128	754	151	B 23
28	621	124	736	147	B 23
31	633	127	758	152	B 25
¹ Feeding started			² B = Controls.		

October 23, when weaned, on October 30, when the addition of anterior lobe or ovarian (desiccated) was made, and also their weights nearly every fourth day thereafter for a period of over three months. The last column shows the average increase of the controls over those fed with anterior lobe.

This table (page 99) and Fig. 2 show that although the groups weighed the same at the commencement of the feeding (1 gm. difference), the controls made a steady gain until the end, the control rats then averaging 25 gm. more than the anterior fed.

The increase in average weight in per cent of original average weight is as follows:

	Anterior.	Control.
First week (normal diet)	37%	41%
December 10	220%	252%
Jan. 31 (whole period)	370%	463%

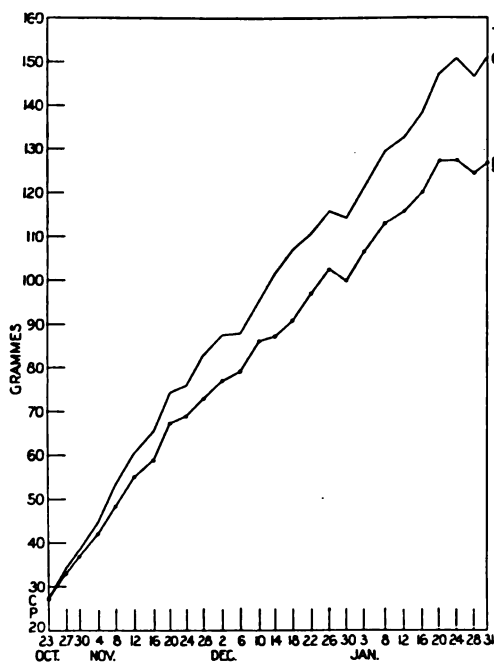


FIGURE 2. — From the table on page 99.

During the feeding the anterior-fed group consumed 7472 gm. of bread and milk mixture (12.6 gm. anterior powder), the controls 7771 gm. (13 gm. desiccated ovary or testicle), or a difference of 299 gm. of bread and milk in favor of the latter.

The chart shows the rate of growth of the two groups of rats. Group A, dotted line, received the addition of anterior lobe of pituitary to food; group B, plain line, the controls, received desiccated ovary or testicle. Addition was made October 30. The dates are marked

upon the abscissa at proportionate intervals, while the weights are given as ordinates.

CONCLUSIONS.

1. The daily ingestion of 30 mg. of fresh desiccated *posterior lobe* of the pituitary of the ox with the food of young white rats does not apparently stimulate their growth, as shown by their weight. Neither is their growth retarded by such feeding.

2. The daily ingestion of 30 mg. of fresh desiccated *anterior lobe* of the pituitary of the ox with the food of young white rats certainly does not stimulate; in fact, it seems in these experiments to have impeded their growth.

The poor showing made by the anterior-fed rats may be explained in part by the fact that this group contained only three against four males in the control group, the males being heavier in every instance. This explanation seems plausible in view of my previous feeding experiments with the anterior lobe where young pups were employed. In this case no pronounced difference was noted between the two groups, and the conclusion was drawn that feeding the anterior lobe does not at least impede their growth.

NOTE. — When the above article was ready for publication my attention was called to an article by Dr. Joseph L. Miller, Chicago, on the "Effect on growth of feeding anterior and posterior lobe of the hypophysis." Dr. Miller employed young white rats, and carried out his experiments in much the same manner as the above. His results were completely negative, both as regards weight and skeletal changes, as shown by the X-ray. No disturbances were noted, the animals thriving in the same manner as the controls.

THE PLACE OF INCIDENCE OF REFLEX FATIGUE.

By ALEXANDER FORBES.

[From the Laboratory of Physiology in the University of Liverpool.]

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I. INTRODUCTION.

IT has been shown by Sherrington, and more recently by Lee and Everingham, that the apparent fatigue of a reflex through prolonged stimulation is not a true fatigue of the moto-neurones employed in the reflex. Sherrington¹ has shown that "when the scratch-reflex elicited from a spot of skin is fatigued, the fatigue holds for that spot, but does not implicate the reflex as obtained from the surrounding skin." The apparent fatigue is regarded by him as synaptic fatigue. Lee and Everingham² infer from this and from similar findings of their own that under such conditions the bodies of the moto-neurones are not fatigued; they term the apparent fatigue "pseudo-fatigue."

It is of interest to know to what extent the apparent fatigue induced in a reflex through one afferent nerve influences the reflex response obtained by stimulating another allied afferent nerve. In a paper on

¹ SHERRINGTON: Integrative action of the nervous system, 1906, p. 218.

² LEE and EVERINGHAM: this Journal, 1909, xxiv, p. 384.

"Reflex inhibition of skeletal muscle"³ I have referred to experiments yet to be described, showing that "extreme fatigue of the flexion reflex as produced through the peroneal nerve rarely impairs the flexion reflex as produced through the popliteal; and *vice versa*." It is the object of the present paper to describe these experiments in detail.

II. METHOD.

The method employed in these experiments was in general the same as that already described in connection with earlier experiments.⁴ Briefly, it was as follows:

The animals (cats) were in all cases decerebrated, the operation being performed under deep ether-chloroform anæsthesia. In some cases decerebration was preceded by low spinal transection at the level of the last thoracic vertebra. After decerebration all the nerves of importance in both hind limbs were severed, except the motor nerve supplying the muscle to be used as an indicator. Experiments were made with both flexor and extensor preparations. The flexor muscle used was the semitendinosus together with that part of the biceps lying adjacent to it; the extensor muscle was the vasto-crureus. Those afferent nerves which were required for stimulation were ligated before severance, to facilitate their handling. The following were employed in different experiments: the peroneal, the popliteal, the sciatic (*i. e.*, peroneal and popliteal combined), and the saphenous.

In all cases the femur was fixed by drills or drill and clamp, and in all but a few of the earlier flexor preparations the muscle was left undisturbed and its contraction recorded by motion of the tibia, a thread being sewed into the leg about an inch below the knee and led over a pulley to the myograph lever. In the earlier flexor preparations the tendon of the semitendinosus was dissected out and attached to the thread. This procedure seemed to impair severely the vitality of the muscle, since after a moderately long experiment scarcely any response could be obtained from it by a direct stimulus sufficient to produce powerful contractions in adjacent muscles. For this reason the muscle was left in all subsequent experiments with its natural connections.

³ FORBES: Quarterly journal of experimental physiology, 1912, v, p. 184.

⁴ FORBES: *Loc. cit.*, p. 163.

Stimulation. — Stimulation was effected by means of two induction coils, whose primaries were connected in series with two accumulators, a resistance box, and a circuit breaker run by a motor. The coils were calibrated empirically in accordance with some of the principles laid down in Martin's papers.⁵ The calibration was carried out as follows: The secondary coil was connected with a frog's gastrocnemius muscle in a moist chamber. With many values of the primary current the positions of the secondary coil yielding threshold stimulation of the muscle were recorded, stimulation being effected by interrupting the primary with the circuit breaker. A curve was plotted with current strength in the primary as abscissæ and distance between coils as ordinates. The procedure was repeated several times with each coil. The resulting curves differed from each other (aside from experimental error) by constants representing the irritability of the individual muscles. By multiplying in each case the current strength by that constant which most nearly gave coincidence with the first curve, an average curve was ultimately obtained. A less careful effort was made to bring the coils in relation with each other, since the observations of Gildemeister and Martin⁶ show that the relation between the stimuli from two dissimilar coils is not constant, but depends on the resistance of the secondary circuit. Accurate comparisons between the stimuli from the two coils were not sufficiently important to warrant determination of the secondary resistance.

By plotting the reciprocals of the current strengths against the distances between primary and secondary coil, a curve was obtained of constants corresponding with " $\frac{M}{L}$,"⁷ by which to multiply the current strength in order to determine the value of a given stimulus. To render the scale thus obtained as near as possible to Martin's scale, the threshold values for the flexion reflex in a number of preparations were compared with those of a number of similar preparations recorded and furnished me by Mr. E. L. Porter. Comparison was also made of the threshold values for the crossed extension reflex with those I had previously obtained at Harvard by the same method with a coil calibrated by Dr. Martin. In this way I was able to make

⁵ MARTIN: this Journal, 1908, xxii, pp. 61, 116.

⁶ See MARTIN: this Journal, 1911, xxviii, p. 49.

⁷ MARTIN: this Journal, 1908, xxii, p. 119.

my empirically obtained scale of units approximate fairly to Martin's scale, and I believe the approximation is within 20 per cent to 25 per cent. The relative values of stimuli from a single coil in different positions are probably correct within a far closer approximation than that, and in these relative values alone was accuracy desired.

Since local polarization of the stimulated nerve must tend to confuse the evidence of central fatigue, and might even lead to cessation of activity before the development of central fatigue, it was important to minimize such polarization as far as possible. To this end a commutator was constructed which, being introduced into the secondary circuit, delivered to the nerve only break shocks, and those in alternately opposite directions through the nerve. The commutator consisted of an ebonite shaft at one end of which was the circuit breaker, a device in which two raised quadrants of ebonite moved a lever and thereby made and broke contact between platinum points in the primary circuit (Fig. 1). The two wires from the secondary coil were led to brushes which made contact with two complete brass collars on the ebonite shaft. Each of these collars was connected with a brass quadrant let into the ebonite. Two brushes on opposite sides of the shaft made contacts with the brass quadrants at the phase of the shaft's revolution in which the break in the primary occurred, and with the intervening ebonite quadrants at the make. The shaft bearing the circuit breaker and the commutator was driven by a motor and made from 30 to 50 interruptions a second, consequently delivered 15 to 25 ascending break shocks a second.

As in previous experiments, platinum stimulating electrodes were placed on opposite sides of the nerve and twisted till the line between them nearly coincided with the axis of the nerve.

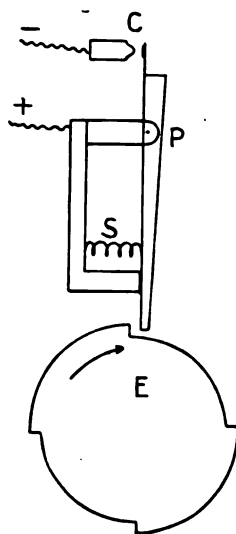


FIGURE 1. — Diagram of circuit breaker. *E*, rotating ebonite shaft with raised quadrants; *S*, spring holding lever against stop; *P*, pivot of lever; *C*, contact between platinum points.

III. PRELIMINARY EXPERIMENTS.

A study was first made of the relative importance of local polarization and central fatigue in causing the observed dwindling of contraction during continued stimulation. Comparisons were made of the fatigue curves of the flexion reflex when the nerve was stimulated

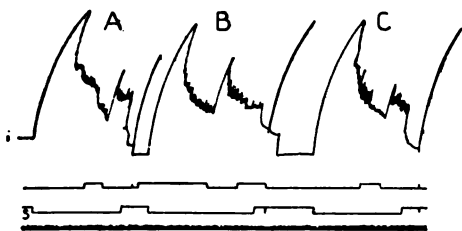


FIGURE 2. — One third the original size. Experiment with non-polarizable electrodes (Preparation 1). Ascent of the myograph line means contraction of flexor muscle. The application of stimuli is marked by a fall in the lower signal line. The upper signal line shows times of shift from one pair of electrodes to the other (see text).

through non-polarizable electrodes and through platinum electrodes. Similar comparisons were made between the curves obtained when the nerve was connected directly with the secondary coil, and when it was connected through the commutator.

In Preparation 1, a flexor preparation with a low spinal transection, the peroneal nerve was dissected out for a considerable length and used for stimulation. At first it was connected with two pairs of non-polarizable electrodes consisting of J-shaped tubes, filled with zinc sulphate and the lower ends plugged with kaolin soaked in Ringer solution. The nerve was laid across the kaolin plugs. The two pairs of electrodes were connected with a rocking device whereby the stimulation could be shifted instantly from one pair of electrodes to the other.

Fig. 2 shows three consecutive experiments with this preparation (No. 1). In *A* the stimulus was applied first to the proximal pair of electrodes, and when the response after dwindling had almost ceased to dwindle, the stimulus was shifted to the distal pair of electrodes, the time of shift being indicated by the rise in the upper signal line; at the fall in the upper signal line the stimulus was shifted back to the proximal electrodes. In *B*, after a rest of four minutes following *A*, the stimulus was applied first to the distal electrodes, then shifted, as indicated by the fall in the upper signal line, to the proximal electrodes, and again at the rise in the signal line back to the distal electrodes. In *C*, after another rest of about three minutes, the stimulus was again applied and shifted as in *A*.

Although the external factors (M , I , L)^{*} in the stimulus were the same whether the distal or proximal electrodes were employed, yet the internal conditions, tissue resistance and kathode surface, could not be identical. Martin has shown that these conditions determine in part the physiological strength of an induction shock. A difference was therefore to be expected between the strength of the stimulus when applied to the proximal and that when applied to the distal pair of electrodes. This difference is shown by the difference in the initial heights of the myograph line occurring in the three consecutive tests *A*, *B*, and *C* (Fig. 2), *A* and *C* representing the proximal electrodes, *B* the distal. It is evident that the stimulus was more intense when delivered by the proximal electrodes than by the distal. It is notable that in *A*, when the stimulus was shifted from proximal to distal, a further decrease in the contraction followed. This fact is capable of two interpretations: (1) The initial decline of contraction is due to central fatigue, and when in *A* the strong proximal stimulus is replaced by the weaker distal stimulus the response is proportionally lowered. (2) The initial decline of contraction is due to local polarization affecting both irritability and conductivity in the stimulated region of the nerve, and the further decrease of contraction following the shift from proximal to distal in *A* is the result of electrotonic block. Thus *A* and *C* furnish no unequivocal evidence of central fatigue. In *B*, however, there is clear evidence that some degree of central fatigue has occurred. When the stimulus is shifted from the distal to the proximal electrodes, there is a marked increase in the contraction, which may be due to the utilization of a fresh portion of the nerve, or to the fact that the proximal stimulus is stronger than the distal, or to both causes acting together. But if the observed decline of contraction were wholly due to local polarization, the contraction following the shift from distal to proximal would be as great as the initial contractions in *A* and *C*, which were evoked through the same electrodes when the preparation was rested. That the contraction following the shift is much less than these initial contractions is obvious, and the amount of reduction is the measure of central fatigue.

Fig. 3 from the same preparation shows the effect of the commutator in reducing local polarization. In the experiment illustrated by Fig. 3

* M = mutual inductance; I = primary current; L = self-inductance. (See MARTIN: this Journal, 1908, xxii, p. 119.)

A, the commutator was in the secondary circuit as before, the procedure was similar to that in the case of Fig. 2 *B*. Fig. 3 *B* shows the result of the same procedure nine minutes later, with the commutator

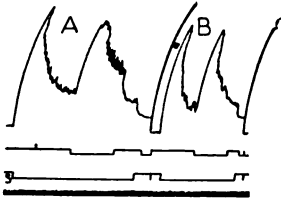


FIGURE 3. — One third the original size. Flexor muscle as in Fig. 2 (Preparation 1). Commutator removed between *A* and *B*.

removed and the secondary coil connected directly with the distributing rocker. Two differences are evident between the two experiments: in *B* the contractions dwindle much more rapidly than in *A*, and the contraction following the shift to the proximal electrodes is more nearly the same magnitude as the initial contraction than in *A*. Both features in *B* show that a local change has contributed to the decline of the response in larger measure than was the case in *A*. But it is not proved that local

change was absent in *A*, only that the commutator had been effective in reducing it.

Soon after the experiment shown in Fig. 3 the contractions became much feebler and almost disappeared. The nerve was then removed from the non-polarizable electrodes and washed in warm Ringer solution. Two pairs of platinum electrodes were then applied to the nerve and secured in position by the method described by Sherrington,⁹ *i. e.*, the electrodes being driven through a rubber stopper fitted in a side branch of a glass tube containing the nerve.

With this arrangement much weaker induction shocks sufficed to produce a maximal reflex than those required with the non-polarizable electrodes, even when the preparation was fresh. Fig. 4, showing the first two experiments with the platinum electrodes, is instructive. Although weaker induction shocks were used here, the initial contractions were greater than any of those obtained with non-polarizable

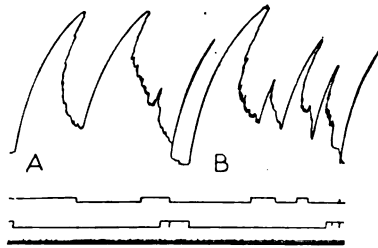


FIGURE 4. — One third the original size. Experiment with platinum electrodes; otherwise the same as Fig 2 (Preparation 1).

⁹ SHERRINGTON: *Journal of physiology*, 1909, xxxviii, p. 382. Cf. FORBES: *Quarterly journal of experimental physiology*, 1912, v, p. 164.

electrodes (Figs 2 and 3); in short, the stimuli as delivered by the platinum electrodes were physiologically stronger than with non-polarizable electrodes. In the experiment recorded in Fig. 4 *A* the stimulus was first applied to the distal pair of electrodes, and, at the fall in the upper signal line, shifted to the proximal pair. The contraction following this shift is seen to be greater than the initial contraction, a result to be contrasted with that of similar shifts with non-polarizable electrodes (Fig. 3, *A* and *B*). The shift back to the distal electrodes shown in Fig. 4 *A* is followed by a slight transient increase in contraction. *B* (Fig. 4) shows the result of the procedure employed in *A* reversed. The stimulus was applied first to the proximal electrodes, then shifted four times, as shown by the upper signal line, to distal, to proximal, to distal, to proximal.

The important facts to be derived from comparing Fig. 4 with Fig 3 are as follows: (1) Central fatigue was evidently a smaller factor in the decline of reflex contraction with the platinum than with the non-polarizable electrodes. This is shown by the fact that the second summit in the myograph in Fig. 4 *A*, resulting from shifting to the proximal electrodes, is scarcely lower than the initial summit in *B*, produced by stimulating through the same proximal electrodes when the preparation was rested. The decline in contraction during prolonged stimulation is therefore due to a local effect on the nerve in larger measure than was the case with the non-polarizable electrodes. (2) Each of the three shifts from proximal to distal electrodes shown in Fig. 4 produced an increase in reflex contraction. Every similar shift with non-polarizable electrodes produced, if any change, a decrease in contraction. It was pointed out above that such a decrease (following a shift from proximal to distal electrodes) might conceivably be explained either as the result of changing from a stronger to a weaker stimulus in case local effects are negligible, or as the result of electrotonic block in case local effects are important. The fact that with platinum electrodes a similar shift caused an increase instead of a decrease in the contraction shows that the former was the chief if not the sole cause, and that the degree of electrotonic block was insignificant at most. For the first fact deduced from the comparison of Figs. 3 and 4 was that local polarization was greater and played a larger part in causing the decline of reflex contraction with platinum than with non-polarizable electrodes. If electrotonic block were the cause

of the decrease in contraction on shifting from proximal to distal electrodes with non-polarizable electrodes, then with platinum electrodes, with which the local effect is more marked (the electrotonic block being greater), the decrease in contraction should also be greater. Since, instead, the decrease is replaced by increase, it is reasonable to

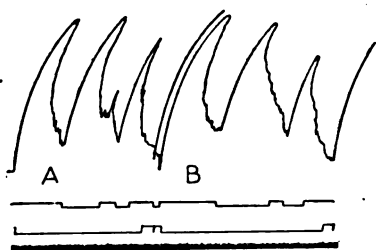


FIGURE 5. — One third the original size.
Procedure as in Fig. 4. Commutator
introduced in B (Preparation 1).

infer that with the platinum electrodes the decline of contraction has been chiefly due to local polarization reducing irritability more than conductivity. An increase in central stimulation follows the change to a fresh portion of the nerve in spite of the fact that the polarized portion lies in the conducting path. In short, although polarization is greater with platinum electrodes, yet shifting to a more distal pair increases the reflex

response. This shows that electrotonic block is slight even when polarization is marked; therefore with non-polarizable electrodes, producing little local polarization of the nerve, electrotonic block must be negligible. Since the decrease in contraction following the shift from proximal to distal non-polarizable electrodes (Fig. 2) cannot be explained by electrotonic block, it follows that the decline of contraction is chiefly due to central fatigue, and the further decline following the shift results from changing to a weaker stimulus. The magnitude of contraction is in this case an approximately true measure of central fatigue.

Fig. 5 shows a comparison of fatigue curves with platinum electrodes with and without the commutator. *A* shows a repetition of the experiment of Fig. 4 *A*, made four minutes after the experiment of Fig. 4 *B*. The same platinum electrodes were connected directly with the secondary coil. *B* shows the result of the same procedure six minutes later than *A*, with the platinum electrodes connected through the commutator, as were the non-polarizable electrodes in the experiments of Figs. 2 and 3 *A*.

Three facts here are noteworthy: (1) After each summit the decline of contraction is slower with the commutator than without. (2) The second summit in *A*, following the shift from distal to proximal elec-

trodes, is higher than the first summit. In *B*, with the commutator, the corresponding summit is lower. (3) In *A* the shift from proximal to distal electrodes is followed by a distinct increase in contraction, as is the case in Fig. 4. In *B* the increase in contraction following a similar shift is almost imperceptible.

All three of these facts seem to indicate that the commutator has been effective in reducing local polarization. In the first place, the slower decline of contraction seems to show that some factor which promotes the decline has been removed, at least in part. In the second place, the fact that shifting to proximal electrodes causes less contraction with the commutator than without argues for a greater degree of central fatigue. Finally, the disappearance of the evident increase of contraction which followed the shift to distal electrodes when polarization was considerable, shows a greater degree of central fatigue and a smaller part played by polarization in causing the decline of contraction.

This series of preliminary experiments indicates that when platinum electrodes are used and are connected directly with the secondary coil, faradization causes considerable local polarization in the stimulated nerve, and this may prevent the development of any great amount of central fatigue. But either the introduction of a commutator, such as that described above, or the substitution of non-polarizable electrodes, reduces the local polarization. With the commutator and the non-polarizable electrodes the least degree of polarization is produced and the observed decline of contraction is almost wholly due to central fatigue.

Since the non-polarizable electrodes are inconvenient to use, and impracticable for a prolonged series of tests, platinum electrodes were employed in all subsequent experiments, and the commutator was regularly used to minimize polarization.

A simple method of testing for central fatigue with two pairs of electrodes on the afferent nerve is the following: The stimulus is sent in through the proximal pair of electrodes just long enough to show the magnitude of contraction produced in the rested preparation; this is repeated two or three times at intervals long enough (*e. g.*, fifteen seconds) to insure rest. Thus, in case of irregularities, an average is obtained. Then the fatiguing stimulus is sent in through the distal electrodes for any desired time, and immediately after its cessa-

tion the test stimulus is repeated as before through the proximal electrodes, and again repeated at intervals till the contractions regain their normal magnitude. The magnitude of the test contraction immediately after the fatigue stimulus when compared with the normal magnitude shows the degree of central fatigue in a way that

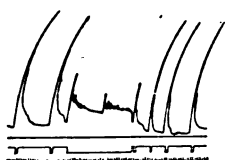


FIGURE 6.—Test of central fatigue. Flexor muscle (Preparation 5). Three pairs of electrodes on peroneal nerve. Stimuli of brief duration applied through proximal electrodes; prolonged stimulus is first with distal, changed while stimulation is in progress to middle electrodes.

cannot be confused by local polarization, since a fresh portion of the nerve is used for all test stimuli (Fig. 6). This procedure was used in all subsequent experiments as a control to show the presence of central fatigue in the reflex arc.

IV. THE RELATIVE INDEPENDENCE OF ALLIED REFLEX ARCS.

Having established a convenient test for central fatigue, I was prepared to ascertain to what extent this fatigue in one reflex arc might influence the activity of allied arcs. To this end three pairs of platinum electrodes were applied to the ipsilateral peroneal nerve in a flexor preparation and one pair to the ipsilateral popliteal nerve. The electrodes on the peroneal nerve were connected with distributing rockers, and thus through the commutator with one secondary coil. The electrodes on the popliteal nerve were sometimes connected directly with the other secondary coil and sometimes with one of the rockers, thus rendering them readily accessible to the coil delivering shocks through the commutator.

After a control experiment had shown that a considerable degree of central fatigue developed after about twenty seconds of continuous stimulation of the peroneal nerve, the effect of thus stimulating the peroneal upon the reflex response obtained from the popliteal nerve was investigated. A submaximal stimulus was applied to the popliteal nerve just long enough to show the magnitude of contraction. This test stimulus was repeated two or three times at intervals to obtain an average. Then a stimulus was applied first to the distal pair of electrodes on the peroneal nerve till contraction had practically ceased, then to the middle electrodes till such renewal of contraction as oc-

curred had practically ceased, and finally to the proximal pair of electrodes. In this way greater central fatigue was insured than could be produced with only one pair of electrodes. Immediately after withdrawal of the stimulus from the peroneal nerve another test stimulus, equal to the earlier ones, was applied to the popliteal nerve, and again repeated at intervals till a resting value was obtained. This procedure was employed with many preparations. In most of these, however, only two pairs of electrodes instead of three were applied to the peroneal nerve.

The results of this procedure were not always the same. In some instances the contractions obtained from the popliteal nerve immediately after the fatiguing stimulation of the peroneal suffered some impairment as compared with those preceding it. In most cases, however, the test contractions following peroneal fatigue suffered no impairment, and in many cases were actually greater after it than before (Fig. 8). In short, the activity of the popliteal reflex arc is largely but not totally independent of fatigue in the peroneal arc. Furthermore, such effect as prolonged stimulation of the peroneal nerve has upon the responsiveness of the popliteal reflex is rather more often improvement than impairment.

The reverse experiment was frequently performed with similar results; that is, the reflex was tested by stimulating the peroneal nerve before and after prolonged stimulation of the popliteal. As in the case of the effect of peroneal fatigue on popliteal response, so in the reverse experiment prolonged popliteal stimulation more often improved than impaired the subsequent peroneal response. In one preparation prolonged peroneal stimulation improved the subsequent response elicited from the popliteal, but popliteal stimulation regularly impaired the subsequent response from the peroneal (Fig. 7). With this preparation only one inductorium was used and connected with electrodes on either nerve at will by rockers; thus all faradization passed through the commutator. In no other preparation was this contrast clearly obtained, and I believe it is an exceptional and not a normal result.

The fact that stimulating one reflex arc even to the point of marked



FIGURE 7. — Flexor muscle (Preparation 3). Brief test stimuli through peroneal nerve. Prolonged stimulus through popliteal nerve.

fatigue, instead of imparting some degree of fatigue to the allied reflex arc, actually improved the subsequent response of the latter, was an

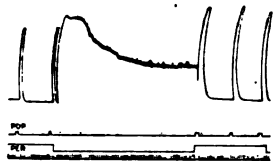


FIGURE 8.—Flexor muscle (Preparation 9). Rise in upper signal line shows stimulation of popliteal nerve. Fall in lower signal line shows stimulation of peroneal nerve through two pairs of electrodes (see text). Commutator in circuit with peroneal nerve only. About one third the original size.

unexpected and surprising result. This result was observed both in the preparations that were simply decerebrate and in those having a low spinal transection as well. The only distinctive difference noted between the two types of preparation was that central fatigue developed more rapidly in the low spinal than in the decerebrate preparation.

Improvement of the reflex obtained through one nerve does not necessarily depend on reflex fatigue obtained through the other nerve. In one preparation which showed improvement of reflex response as obtained through the popliteal nerve when

fatigue had been induced through the peroneal (Fig. 8), and *vice versa* (Fig. 9), it was found that similar results followed stimulation too brief to fatigue the reflex arc (Figs. 10 and 11).

V. SIMILAR INDEPENDENCE IN THE CASE OF INHIBITION.

Sherrington has pointed out the close correspondence in many features of reflex excitation and reflex inhibition.¹⁰

It is an interesting question whether the relative independence of allied reflex arcs appears in inhibitory as well as in excitatory activity. I have already shown fatigue in the inhibitory reflex arc taking part in the flexion reflex;¹¹ that is, I have shown that prolonged inhibition of the knee extensor through an ipsilateral afferent nerve impairs for a time the inhibitory power of the arc employed. Does this fatigue affect the inhibitory centre as a whole, or does it affect merely the particular



FIGURE 9.—Preparation 9. Arrangement as in Fig. 8.

¹⁰ SHERRINGTON: Proceedings of the Royal Society, 1905, lxxvi, B, p. 269; also the Quarterly journal of experimental physiology, 1903, i, p. 67.

¹¹ FORBES: Quarterly journal of experimental physiology, 1912, v, p. 179.

channel of approach to the centre, as in the case of the excitatory phase of the same reflex?

To answer this question the vasto-crureus preparation was used; the sciatic nerve of the opposite hind leg was stimulated in order to produce the requisite background of knee extension for the demonstration of inhibition. The ipsilateral popliteal and peroneal nerves were prepared for inhibition, and separate pairs of electrodes were applied as in the experiments on reflex

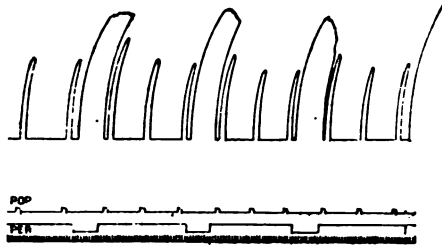


FIGURE 10. — Preparation 9. Arrangement as in Fig. 8.

contraction, two pairs on the peroneal and one pair on the popliteal.

The experiments were conducted as follows: To control the question of polarization and prove central inhibitory fatigue, extensor contraction was induced and then partly inhibited by moderate stimulation of the peroneal nerve through the proximal pair of electrodes. After several repetitions of the tests at intervals to show the average degree of inhibitory relaxation thus obtained, a prolonged inhibitory stimulus was applied through the distal pair of electrodes on the peroneal nerve.

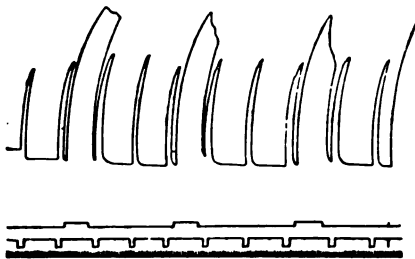


FIGURE 11. — Preparation 9. Arrangement as in Fig. 8.

As soon as this ceased, the test of inhibition was applied again through the proximal electrodes with the same strength of inhibitory stimulus as before. If reduction of inhibitory relaxation resulted, showing that the prolonged inhibition had sufficed to produce fatigue, its effect on inhibition as produced through the popliteal nerve

was then tested. For this the experiment was repeated with the test inhibitory stimuli applied through the popliteal nerve, the fatiguing inhibitory stimulus being applied as before through the peroneal nerve. This and the reverse experiment (*i. e.*, the effect of popliteal inhibition on peroneal) were both tried many times.

The results were strikingly similar to those recorded above, relating to the excitatory reflex. Inhibition which sufficed to impair subsequent inhibition through the same nerve

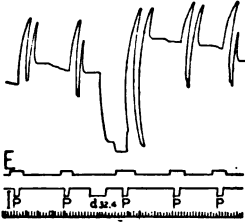


FIGURE 12.—Extensor muscle. Ascent of the myograph line shows contraction. Rise in upper signal line shows excitatory stimulus (crossed sciatic nerve). Fall in lower signal line shows inhibitory stimulus, marked *P* when applied to popliteal nerve, *d* when applied to peroneal nerve. One third the original size.

usually improved subsequent inhibition through the other nerve. Fig. 12¹² shows a case of improved popliteal inhibition following prolonged peroneal inhibition. Fig. 13 shows a similar improvement of peroneal inhibition after popliteal inhibition. As in the case of the excitatory reflex, exceptions occurred wherein the test inhibition through the popliteal nerve was impaired after prolonged peroneal inhibition, and *vice versa*, but these were unusual.

From these observations it is evident that the allied reflex arcs whose afferents are the popliteal and peroneal nerves and whose motor effects are knee flexion are so related, both with respect to excitation of flexors and inhibition of extensors, that, while relatively independent of each other, activity in one arc modifies subsequent activity in the other. And yet the modification varies, sometimes even in a single preparation, appearing usually as improvement, but occasionally as impairment of reflex activity.

VI. THE EFFECT OF VARYING THE STRENGTH OF STIMULATION.

It seemed important to ascertain, if possible, the cause of the variation observed in the influence of activity in a reflex arc on subsequent activity in an allied arc. Evidence was found in an experiment with the inhibitory reflex which seemed to indicate that the strength of the stimulus played some part in determining whether the subsequent activity of the allied reflex arc was impaired or improved. Increasing the strength of the stimulus above a certain

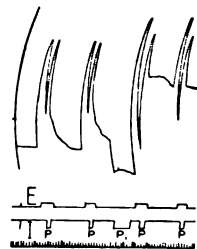


FIGURE 13.—Arrangement as in Fig. 12. *P* means inhibitory stimulus applied to peroneal nerve, *P*₁ to popliteal nerve.

¹² In Figs. 12, 13 and 14 the kymograph was stopped about thirty-five seconds during prolonged stimuli to save space on the drum.

point appeared to cause the usual improvement to be replaced by impairment. Several experiments were performed with both excitatory (flexor) and inhibitory (extensor) preparations with a view to determining whether this was regularly so. Variations in the reflex condition of the preparation and certain defects in the apparatus rendered it difficult to obtain uniform evidence, but a majority of the observations corroborated the first indications. The results of the experiments bearing on this point may be summarized as follows.

In the case of flexor preparations, showing the excitatory phase of the reflex, five were tested for the effects of different strengths of peroneal stimulation upon subsequent reflex activity obtained from the popliteal nerve. Four of these yielded positive results; that is, with moderate peroneal stimuli the contraction obtained from the popliteal was greater afterwards than before, with stronger peroneal stimuli the popliteal contraction was no greater, or even smaller afterwards than before. The fifth preparation showed apparent improvement of popliteal response after peroneal stimulation of all strengths; but this apparent improvement may not have been real, since the muscle remained shortened after the cessation of each peroneal stimulation in this preparation, and the subsequent test contraction, therefore, started from a higher base line than the initial test contraction. The evidence furnished by this preparation was therefore uncertain.

Seven preparations, including four of the five just discussed, were examined for the effects of different strengths of popliteal stimulation upon subsequent activity induced from the peroneal nerve. Two of these showed results opposite to the observed tendency; that is, after moderate popliteal stimulation the peroneal response was impaired, and after stronger popliteal stimulation it was improved. But in at least one of these two preparations the results were of little significance, since a considerable time elapsed between the tests,—enough for marked alteration in the reflex state to have occurred. One preparation showed improvement of peroneal response following popliteal stimulation of the only two strengths employed; another showed impairment following the only two strengths employed. Three preparations in which a fair range of stimulation strengths was tested showed positive results with some regularity. There seemed to be in each a critical value of stimulation in the popliteal arc below which the effect was to improve subsequent peroneal response and above which the effect was to impair it.

In the case of extensor preparations, showing the inhibitory phase of the flexion reflex, four preparations were examined for the influence of the strength of stimulus in determining the effect of peroneal stimulation on subsequent popliteal response. Two of these showed impairment of popliteal inhibition following all strengths of peroneal inhibition employed. The other two preparations yielded positive results with marked regularity; that is, popliteal inhibition was greater after a period of moderate peroneal inhibition than before it, but was less after strong peroneal inhibition than before.

Three of these same preparations were examined for the influence of different degrees of popliteal inhibition on subsequent peroneal inhibition. One showed improvement of peroneal inhibition after popliteal inhibition of all strengths from 10 to 57 stimulation units. The other two preparations yielded positive results regularly corresponding with those recorded above. Critical values appeared in both, below which popliteal inhibition improved subsequent peroneal inhibition and above which improvement was replaced by impairment.

From these observations it seems that in a majority of cases increasing above a certain critical value the strength of stimulus applied to either popliteal or peroneal nerve changes the effect of such stimulation upon subsequent activity induced through the other nerve from one of improvement to one of impairment. And this is true of both the excitatory and the inhibitory phases of the flexion reflex. That the strength of stimulus tends to modify the result in this way seems to me fairly clear. The lack of uniformity of the results may be due to fortuitous experimental error, or to changes in the reflex state of the preparation or in the condition of the peripheral nerves working corresponding changes in the central value of a given peripheral stimulus, or to other factors not accounted for entering into some tests more than others. The method is quantitatively crude at best, and perfect uniformity cannot be expected with it.

VII. THE EFFECT OF VARYING THE DURATION OF THE STIMULUS.

If changes in the strength of stimulus modify the effect of activity in one reflex arc upon subsequent activity in an allied arc, changes in duration might be expected to produce similar modification; for duration as well as strength determines the total amount of stimulation

inflicted on the centre. A few experiments in changing the duration of the stimulus were tried with flexor preparations. It has already been stated that in one preparation brief excitation through one nerve produced as marked an improvement in subsequent activity induced from the other nerve as excitation prolonged to the point of fatigue (Figs. 10 and 11). In another preparation the influence of duration was examined as follows: A series of brief uniform test stimuli were applied to one nerve at intervals of from fifteen to twenty seconds. Just before every third test stimulus the other nerve was stimulated, and the duration of these intercurrent stimuli was varied from one second to fifteen seconds. The results of this procedure were quite irregular and showed little constant influence of duration. Increase in the magnitude of contraction usually followed intercurrent stimulation of the other nerve, and on the whole this seemed to be slightly less after prolonged than after brief stimulation. But this impression must not be relied upon. The irregularity in the contractions was probably due to variations in the strength of the stimuli resulting from a defect in the working of the commutator, already referred to in an earlier paper.¹³

VIII. RELATION OF OBSERVATIONS TO THOSE ON "CRITICAL VALUE OF INHIBITION."

I have shown elsewhere¹⁴ that in the knee extensor preparation the effect of prolonged reflex inhibition upon subsequent reflex excitation depends on the strength of the inhibitory stimulus. With moderate stimuli the inhibition favors subsequent activity, with strong stimuli it depresses it. The intermediate strength of inhibitory stimulus which causes neither "subsequent augmentation" nor "subsequent depression" of excitatory activity I termed the "critical value of inhibition." The existence of a somewhat similar critical value relating to the effect of reflex stimulation on the subsequent activity of an allied as well as on that of an antagonistic reflex arc is interesting. In one extensor preparation in which the "critical value of inhibition" was fairly marked there was noted following popliteal inhibi-

¹³ FORBES: Proceedings of the Royal Society, 1912, lxxv, B, p. 296.

¹⁴ FORBES: Quarterly journal of experimental physiology, 1912, v, p. 173.

tion with 41 units, just below critical value, in addition to the increase in extension ("subsequent augmentation"), an increase in subsequent peroneal inhibition (Fig. 14 A); about a minute later popliteal inhibition with 46 units, just above critical value, was followed not only by "subsequent depression" of excitatory activity, but also by a decrease in subsequent peroneal inhibition (Fig. 14 B). In this case, then, the two critical values practically coincided, both lying between 41 and 46 units. At 41 units popliteal inhibition caused improvement in the subsequent activities of both the antagonistic excitatory arc of the crossed extension reflex and the allied inhibitory arc of the flexion reflex with the peroneal afferent; at 46 units popliteal inhibition caused impairment of the subsequent activities of both the

excitatory arc and the allied inhibitory arc.

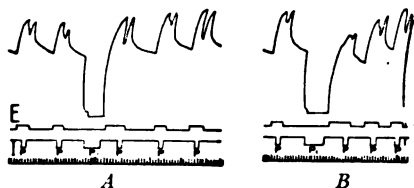


FIGURE 14. — Arrangement and notation as in Fig. 13. Popliteal stimulus lasted 45 seconds in each case.

It was impossible to determine accurately this relationship between the two critical values in many preparations. Two preparations, besides that just mentioned as showing approximate coincidence of critical values,

were available for similar comparisons. The first of these showed a distinct lack of coincidence throughout the experiment, sometimes showing "subsequent augmentation" in the same test with impairment of inhibition in the allied arc, and sometimes "subsequent depression" in the same test with improvement of this inhibition. When the "critical value of inhibition" (*i. e.*, with respect to subsequent excitation) was demonstrable, it lay between 32 and 58 units, when applied through the peroneal nerve. When the critical value at which the after-effect of peroneal inhibition on subsequent popliteal inhibition changed from improvement to impairment was demonstrable, it lay between 9.5 and 11.5 units. The other preparation showed an approach to coincidence between the two critical values. The critical value for subsequent excitation lay between 24 and 32 units with the peroneal nerve; the critical value for subsequent popliteal inhibition was 32 units or a little above.

IX. EXPERIMENTS WITH SAPHENOUS AND SCIATIC NERVES.

A few experiments were performed similar to those already described, but with the whole sciatic nerve (distal to the branching of the hamstring) as one afferent, and the saphenous nerve as the other. The saphenous nerve when dissected out for stimulation lost its conductivity so rapidly that a long series of tests with it was impossible. But from a few comparatively short experiments results were obtained quite similar to those obtained with the popliteal and peroneal nerves. Stimulation of the saphenous nerve caused little or no change in the flexion reflex subsequently obtained from the sciatic nerve, although in one or two instances it appeared to produce a slight improvement.

The effect of sciatic stimulation upon the subsequent response obtained from the saphenous nerve was striking. In the flexor preparation, following stimulation of the sciatic nerve either for a short time or long enough to produce fatigue, there was almost invariably a marked increase in the contraction obtained by

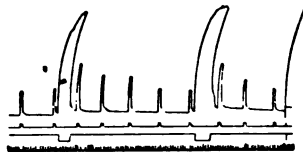


FIGURE 15. — Flexor muscle. Rise in upper signal line shows stimulus applied to saphenous nerve; fall in lower signal line shows stimulus applied to sciatic nerve.

stimulating the saphenous nerve as compared with the contractions previously obtained by the same stimulus (Fig. 15). This increase was usually greater and occurred more regularly than in the case of popliteal following peroneal stimulation or the reverse, and it was never replaced by diminution on increasing the strength of sciatic stimulation, as was frequently the case with the popliteal and peroneal nerves. In one preparation the increase in subsequent saphenous response was as great after sciatic stimulation with 47 units as it was after the sciatic stimulation indicated in Fig. 15 with 14 units.

Sciatic and saphenous inhibition in the extensor preparation were similarly examined. The results were not very satisfactory, but showed little influence of sciatic inhibition on subsequent saphenous inhibition. In one case there was a distinct increase in the subsequent saphenous inhibition comparable to the increase in saphenous excitation in the flexor preparation.

X. CONCLUSION.

The fact that central fatigue induced through one afferent nerve usually does not impair the reflex involving the same muscles induced through another afferent nerve supports the conclusions of Lee and Everingham¹⁴ that this fatigue does not involve the moto-neurones, and accords with the view of Sherrington¹⁵ that its seat is the synapse. The reflex centre is not fatigued as a whole, but merely the particular channel of approach employed. We may suppose that the popliteal and peroneal nerves are connected with the moto-neurones through two independent sets of synapses, and that fatigue in one set does not affect the other. It is interesting in this connection to recall the experiments of Camis¹⁶ showing that maximal stimulation of one afferent nerve does not evoke all the reflex excitation of which the centre is capable.

The fact that activity in one of these reflex arcs which excite the same muscles actually predisposes the other arc to activity is remarkable, and its cause presents an interesting problem. It may conceivably be akin to the familiar "treppe" in muscle. If so, it may be caused by the small quantity of CO₂ generated in the reflex centre by activity, granted that the similar explanation of "treppe" is tenable.

On the assumption that some tendency exists to promote further activity in allied arcs, whether or not akin to "treppe," it becomes interesting to inquire into the significance of the critical value of reflex stimulation above which the resulting improvement is replaced by impairment. Perhaps in the case of two reflex arcs as closely related as those of the popliteal and peroneal nerves there is a slight community of fatigue. From fatigue in one set of synapses an effect may spread to adjacent synapses. On this assumption we should conclude that with a stimulus of moderate strength this spread of fatigue effect is too small to offset the activating effect and impairment results, but that with strong stimuli the spread of fatigue effect is great enough to overbalance the activating effect and impairment ensues. The critical value would then be that strength of stimulus at which the fatigue

¹⁴ LEE and EVERINGHAM: this Journal, 1919, xxiv, p. 384.

¹⁵ SHERRINGTON: Integrative action of the nervous system, 1906, p. 218.

¹⁶ CAMIS: Journal of physiology, 1909, xxxix, p. 228.

effect and the activating effect just balance and leave the allied arc neither exalted nor depressed.

In harmony with this view is the observation that saphenous response is regularly improved after sciatic stimulation and does not suffer impairment when the strength of sciatic stimulation is increased. For since the saphenous nerve is anatomically remote from the sciatic as compared with the proximity of the peroneal and popliteal nerves, we may suppose that its synapses are less accessible to the spread of fatigue effects from those of sciatic than are those of the popliteal to similar spread from those of the peroneal. If, then, we assume that the activating effect is one which influences the centre as a whole (as we should expect if it were due to CO_2), and that fatigue is localized at one set of synapses, its effects being limited to a slight spread only to adjacent synapses, we can consistently explain all the phenomena recorded.

In an earlier paper where I described the "critical value of inhibition" for subsequent excitation of the inhibited moto-neurones, I suggested¹⁷ that "subsequent depression" of excitatory response might be due to the spread of fatigue effects from the inhibitory to the excitatory synapses. It seemed as if stimulation above a certain strength exerted a harmful effect on all the activities of the centre. The fact that in one case, described in section VIII, inhibitory stimulation above the critical value impaired subsequent inhibition through the allied nerve as well as subsequent excitation through the crossed nerve was also suggestive of the same view. Possibly any stimulus of sufficient power, whether inhibitory or excitatory, damages the centre and leaves all its activities depressed for a time.

The fact that in other preparations the critical value for inhibition through an allied nerve did not coincide with the "critical value of inhibition" for excitation seems at first sight to refute the view just proposed. And yet it does not necessarily disprove it. Although the critical values do not coincide, the impairment of the various central activities following strong stimulation may be due to a common cause.

That sometimes each activity has its separate critical value may be because the activating tendency is stronger for one reflex than for another and therefore requires more of the depressing tendency to offset it. For instance, in the preparation showing a "critical value of

¹⁷ FORBES: Quarterly journal of experimental physiology, 1912, v, p. 185.

inhibition" for excitation between 32 and 58 units, and a critical value for inhibition through another nerve between 9.5 and 11.5 units, the activating effect was perhaps much stronger for excitation than for adjacent inhibition, and although the same depressing tendency offset it in both cases, more depressing tendency was required in the former than in the latter. Moreover, other factors as yet unobserved, such as changes in blood pressure, ventilation or other internal conditions, may, and probably do, play a large part in determining these critical values.

One thing seems to me clear, that many conditions now largely inaccessible must be better understood or controlled before the various modifying tendencies inferred in this discussion can be examined and measured with precision.

THE IODINE CONTENT OF THE SMALL, MEDIUM, AND LARGE THYROID GLANDS OF SHEEP,¹ BEEF, AND HOGS.²

By T. B. ALDRICH.

IT is conceded by a majority, if not all, of the writers on the subject of thyroid therapy, that the thyroid gland (or its preparations), to be physiologically active, must contain at least *some* iodine; furthermore, that this iodine, to be of the greatest value therapeutically, must be combined or associated with some protein or organic complex found in the gland. Presumably the iodine is the more important constituent; the two, however, associated or combined, seem to give the best therapeutic results, and to-day the efficiency of a thyroid preparation is generally measured, or should be, by its iodine content. In fact, for some time a number of pharmaceutical houses have been putting out thyroid preparations with a guaranteed percentage of iodine. Since then the iodine content of a thyroid preparation is a measure of its therapeutic efficiency, it is desirable to select, if possible, those glands which contain the most iodine, providing other factors are equal, and from those animals whose thyroids contain the most of this constituent.

The following work was taken up with the object of determining the iodine content, and thereby the therapeutic efficacy, of some thyroid glands, especially the small, medium, and large thyroid glands of sheep, beef, and hogs, by means of the method employed by Hunter,³ which is very accurate and detects the presence of very small amounts of iodine, that are incapable of being detected by the older method

¹ The iodine content of some mixed sheep thyroids was also determined.

² Read before the Eighth International Congress of Applied Chemistry, Washington and New York, September 4 to 13, 1912.

³ HUNTER: Journal of biological chemistry, 1909-1910, vii, p. 321.

of Baumann,⁴ which has been the method usually employed heretofore.⁵

The glands received came from the Chicago stockyards, were placed in Mason jars as soon as removed from the animals, subsequently placed in cold storage and shipped packed in ice. They were all received in the best possible condition. Six lots were obtained as follows:

1. Mixed sheep thyroids (Lots A and B).
2. Small, medium, and large sheep thyroids (Lot C).
3. Small, medium, and large sheep thyroids (Lot D).
4. Small, medium, and large beef thyroids (Lot E).
5. Small, medium, and large hog thyroids (Lot F).

In lots A, B, and C the glands were not counted.

After freeing from superfluous tissue and weighing, the glands were ground very fine, defatted, and desiccated in the usual manner, and eventually reduced to a very fine powder by passing through a 60-mesh sieve.

The following information obtained by one⁶ of our staff from a packer, relative to thyroids, may be of interest at this point:

1. Sheep thyroids are subject to great variation in size. The sex factor is not the determining factor for the size of the gland, nor has the condition of nutrition of the sheep any decisive bearing on the size of the gland. They run from the size of an almond to the size of a lemon.

2. Steer's thyroids are larger than cow's, this variation in size being a *constant* factor; size of gland varies again with the condition of the animal. Well-nourished cattle have larger thyroids than poorly fed ones,

3. In hogs the thyroids vary little in size, and present only slight variations in general appearance.

The thyroids of cattle are removed after the head has been severed;

⁴ BAUMANN: Journal of physiological chemistry, 1896, xxi, p. 489; *Ibid.*, xxii, p. 1.

⁵ See also RIGG's work, Journal of American Chemical Society, 1910, xxxii, p. 692; *Ibid.*, 1909, xxxi, p. 710.

⁶ It is a pleasure to thank Dr. BAESLACK, of our staff, for looking after the collection of the glands and also for the information relative to the same.

Iodine Content of Thyroid Glands of Sheep, Beef, and Hogs. 127

the same is true of hogs. Cattle thyroids are often cut, those of hogs not.

The following method of assaying the iodine, somewhat abbreviated, was employed:⁷

Exactly 1 gm. of the body was taken, placed in a nickel crucible (125 c.c.), 14 gm. of the following oxidation mixture added (106 parts sodium carbonate, 75 parts potassium nitrate, and 138 parts potassium carbonate), and the two intimately mixed by means of a nickel spatula. Over this was dusted 4 gm. of the oxidation mixture. The nickel crucible was then heated over a flame until the contents of the same was perfectly white. This more or less fused mixture was dissolved in water and brought into an Erlenmeyer flask (800 c.c.). After cooling 35 c.c. of sodium hypochlorite solution was added, and while holding the flask in a slanting position in cold water and agitating at the same time, 65 c.c. of 42½ per cent phosphoric acid was added. The solution should, after the addition of the acid, be colored slightly yellow from the slight excess of chlorine liberated. The mixture was then boiled briskly, a funnel with a short stem being placed in the neck of the flask to avoid any loss. When all the free chlorine was expelled, recognized by holding filter paper moistened with starch solution containing potassium iodine in the steam (blue color if present), the flask, containing now about 70-80 c.c., was allowed to cool and brought up to about 200 c.c. by the addition of water. To this cold solution 10 c.c. of a 1 per cent potassium iodine solution was added, which causes the liberation of six times the amount of iodine originally in the product to be assayed, according to the following equation:



This liberated iodine is immediately titrated with a standard solution of sodium thiosulphate solution approximately N/200, a few drops of starch solution being added toward the end of the reaction.

The number of cubic centimetres of sodium thiosulphate used, multiplied by the iodine factor, then divided by 6, gives the amount of iodine in the original sample.

A blank test using casein, or some other body free from iodine, was

⁷ For details see HUNTER: Journal of biological chemistry, 1909-1910, vii, p. 321.

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made to insure the absence of iodine in the reagents, and the usual precautions employed in analytical methods observed.

The table on page 128 gives the number of glands (except in the lots A, B, and C), total weight of glands, average weight of glands, and iodine content in each lot, with average iodine content and percentage in each gland, where the number of glands is known.

The average iodine content of the mixed sheep thyroid glands, Lots (A), (B), (C), and (D) where over 50 lb. were employed, is about .025 per cent, while in some cases with selected glands .044 per cent has been obtained, and .027 where relatively large amounts (6000 gm.) were used.

The beef thyroids (Lot E) gave an average of .036 per cent iodine, and in selected cases nearly .04 per cent; the hog, .047 per cent average, in selected glands over .05 per cent iodine

It will be noted that the greatest variation in iodine content in the different-sized glands of the same animals is in that of the sheep, where it varies from .01 to .027 per cent in Lot C and from .015 to .044 per cent in Lot D, this variation being, no doubt, due to the greater prevalence of goitre in sheep.

Next to sheep the iodine content of the hogs' thyroids vary the most, .041—.054 per cent, while in the beef we have the least variation, .030—.039 per cent.

The hogs' thyroids⁸ contain the highest percentage of iodine, with the small sheep glands in one case higher, in the other lower, than the mixed beef glands. Assuming the iodine content to be a measure of the therapeutic activity, the mixed thyroids of the hog are superior to the beef and the latter superior to sheep thyroids, small sheep thyroids being about equal to mixed beef thyroids.

Weight for weight, the small glands of all the animals studied, nearly without exception, contain the most iodine (excepting beef, where the large and the small have nearly the same, .038 per cent).

In general, the larger glands contain the most iodine, and the ratio of the iodine content of the small, medium, and large glands is approximately as follows:

In the sheep 2:3:7
In the beef 1:1:2
In the hog 3:4:6

⁸ It is interesting to note that BAUMANN found very little iodine in pigs' and hogs' thyroid glands, very much less than in beef and sheep.

The mixed glands, arranged according to their iodine content, stand about in the following ratio:

Sheep 5

Beef 7

Hogs 9

From whatever standpoint we take we must conclude, from the above, that the employment of either hog or beef thyroids for therapeutic purposes would be more rational than the employment of sheep glands, even if small selected sheep glands are employed, thus eliminating the goitrous glands.

THE EFFECTS OF ALKALOIDS ON THE DEVELOPMENT OF FISH (FUNDULUS) EGGS.

By J. F. McCLENDON.

[From the Embryological Laboratory of Cornell University Medical College, New York City, and the U. S. Bureau of Fisheries, Woods Hole, Mass.]

THE goal of experimental embryology is the control of development, notwithstanding the fact that the majority of attempts in this direction have been failures. The embryo results from the interaction between the egg and its environment, and we might expect that a specific change in the medium would produce a specific change in the embryo. However, the organism is capable, to a great degree, of maintaining constant conditions within itself. Take, for example, the remarkable constancy in body temperature and composition of the blood of mammals.

One mechanism for the maintenance of constant chemical conditions within the organism is evidenced in the remarkable semi-permeability of living cells. Overton found that volatile anæsthetics and free alkaloid bases, which are rarely encountered by cells, penetrate easily, whereas salts, with which cells are constantly in contact, do not ordinarily penetrate. I observed that neither salts nor anions penetrate the *Fundulus* egg, but that kations outside may be exchanged for those within.¹

Herbst² thought he had found a specific effect of lithium salts on sea urchins' eggs in the production of exogastrulæ, *i. e.*, gastrulæ in which the archenteron is evaginated instead of invaginated. However, Driesch³ produced the same results by a rise in temperature to 30° C. In this case the archenteron, or gut, sometimes shrank and disappeared, producing a condition known as anenteria.

¹ McCLENDON: this Journal, 1912, xxix, p. 295.

² HERBST: *Zeitschrift für wissenschaftliche Zoologie*, 1892, lv, p. 442, and *Mitteilungen der zoologische Station zu Neapel*, 1895, xi, p. 136.

³ DRIESCH: *Ibid.*, 1895, xi, p. 221.

Gurwitsch ⁴ supposed that lithium salts produced a radially symmetrical gastrula in the frog's egg. Morgan ⁵ showed this not to be the correct interpretation, but the chief characteristic of these embryos is that the endodermal cells are not invaginated, and hence we might call them exogastrulæ.

In opposition to the above statements, Bataillon ⁶ denies that lithium or other salts or sugar act otherwise than osmotically, and states that isotonic solutions all have the same effect on frog's eggs.

Stockard ⁷ observed that lithium chloride causes an enlarged segmentation cavity, and retards the down-growth of the blastoderm over the yolk, in *Fundulus* embryos. He demonstrated that this is independent of the osmotic pressure of the medium, and in a later paper ⁸ stated that these abnormalities are "specific for the lithium ion in its action on this egg."

On the other hand, I produced "lithium embryos" with sodium chloride, calcium chloride, ether, acetone, and dextrose.⁹

Stockard produced cyclopic or one-eyed *Fundulus* embryos, and at first thought the abnormality due to the specific action of the magnesium ion,¹⁰ but later obtained similar results by the use of volatile anæsthetics, and supposed them due to the specific action of anæsthetics.¹¹

However, I obtained the same results, not only with several indifferent anæsthetics, but with sodium chloride, lithium chloride, and sodium hydrate, which are considered stimulating rather than anæsthetic in their action.¹² I found the order of effectiveness of kations (added to sea water) in producing cyclopia to be $Mg < Li < Na$. Since Hedin ¹³ found the same order in the rate of diffusion of these ions through dead ox gut, we may suppose their action in producing cyclopia probably to be physico-chemical. This may be true also of indifferent anæsthetics, since I showed that their effectiveness in producing

⁴ GURWITSCH: Archiv für Entwicklungsmechanik, 1896, iii, p. 219.

⁵ MORGAN: *Ibid.*, 1903, xvi, p. 691.

⁶ BATAILLON: Archiv für Entwicklungsmechanik, 1901, xi, p. 149.

⁷ STOCKARD: Journal of experimental zoölogy, 1906, iii, p. 399.

⁸ STOCKARD: *Ibid.*, 1907, iv, p. 165.

⁹ MCCLENDON: this Journal, 1912, xxix, p. 297.

¹⁰ STOCKARD: Journal of experimental zoölogy, 1909, vi, p. 285.

¹¹ STOCKARD: American journal of anatomy, 1910, x, p. 369.

¹² MCCLENDON: this Journal, 1912, xxix, p. 289.

¹³ HEDIN: Archiv für Physiologie, 1899, lxxviii, p. 205.

cyclopia is proportional to their effectiveness in lowering the surface tension of water, and also proportional to their toxicity. The concentrations of salts and anæsthetics producing cyclopia are very near the lethal doses, although lower than those producing "lithium embryos."

EXPERIMENTS.

The majority of drugs that have a specific action on the function of parts of the human body are included in the old group of alkaloids. Since the chemistry of many of these substances is unknown, the group cannot be well defined, and we will use the name as originally applied to substances of vegetable origin with basic properties. Many synthetic substitutes of the alkaloids might well be included in the group.

I have tried a number of alkaloids on the eggs of *Fundulus heteroclitus*, and produced the same abnormalities with each of them. Some experiments with glucosides were begun, but were cut short by the unusually early close of the breeding season. Comparative studies were made on the eggs of the sea urchin, *Arbacia punctulata*.

Of the alkaloids tried, caffeine and theobromine are xanthine bases, the remainder being derivatives of pyridine and quinoline. Stovaine (chlorhydrate of dimethylaminobenzoylpentanol), a synthetic product, was also used.

The alkaloids were usually made up in centi-molecular solutions in sea water, and various strengths obtained by dilution. The free base was used except in three cases. The hydrochloride of quinine was chosen. The sulphates of strychnine and morphine were used, and made up of half strength, since each molecule liberates two molecules of the free base. These salts of the alkaloids have the advantage of being more easily dissolved, and since sea water is alkaline, the free base is completely liberated in the solutions. Overton showed that alkaloids enter living cells only in the form of the free base, hence they are effective only in alkaline or neutral solutions.

In the stronger solutions the whole embryo degenerates, but in weaker solutions certain parts are affected more than others. Organs which arise early may degenerate, those which appear later may fail to develop. The circulatory system is the most affected, and may be suppressed to a greater or less extent, or may develop and not function, or may function for a while and then degenerate. The heart and

respiratory capillaries lie upon the surface of the yolk sac, and were more carefully observed than were the vessels which are obscured by the surrounding tissues of the embryo.

FIGURE 1.



FIGURE 3.



FIGURE 4.

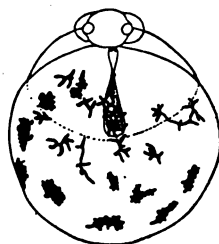


FIGURE 1. — Normal embryo of *Fundulus heteroclitus*, six days old. S, sub-intestinal vein; D, duct of Cuvier.

FIGURE 3. — *Fundulus* egg treated with $\frac{1}{100}$ molecular caffeine, showing the degenerating embryo one week old.

FIGURE 4. — Front view of embryo from $\frac{1}{100}$ caffeine, one week old. The heart beat, but the blood did not circulate.

A glance at the normal embryo will be useful for comparison. Fig. 1 shows the yolk sac circulation in a six-day embryo, the capillaries on the ventral side being indicated by dashed lines. The caudal vein passes on to the yolk sac as the sub-intestinal vein (S), and breaks

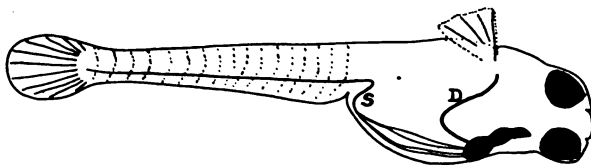


FIGURE 2. — The same embryo as Fig. 1, just hatched.

up into capillaries. The ducts of Cuvier pass out of the embryo and break up into capillaries, which anastomose with those from the sub-intestinal vein. This capillary plexus is reunited at the venous end of the heart, in front of the head of the embryo. It should be noted that in early stages the ducts of Cuvier draw their blood from the dorsal aorta through temporary connections.

As the embryo develops, the yolk is absorbed, and the capillaries on the yolk sac are gradually transformed into three large veins, the continuations of the ducts of Cuvier and the sub-intestinal vein. A transition stage is seen in an embryo just hatched (Fig. 2). The

right duct of Cuvier (*D*) is shown completed, but the sub-intestinal vein (*S*) is connected with the heart by four parallel veinlets, the remains of the capillary plexus. The anastomoses between the sub-intestinal vein and ducts of Cuvier have disappeared.

In the stronger solutions of alkaloids the embryos begin to develop normally, but sooner or later the cells begin to be loosened one from

FIGURE 5.

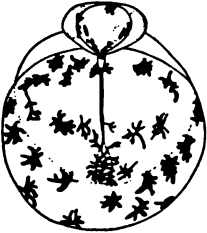


FIGURE 6.



FIGURE 7.



FIGURE 5. — Same embryo as Fig. 4, ten days old.

FIGURE 6. — Embryo from $\frac{1}{100}$ caffeine solution, eleven days old.

FIGURE 7. — Cyclopic monster from $\frac{1}{100}$ nicotine solution, four days old.

another, a condition called by Roux "framboisea." Such an embryo may live a long time, but gradually undergoes de-differentiation.

In Fig. 3, which represents such an egg a week old, the stippled area represents the embryo. The spots with blunt processes represent black chromatophores, and those with slender processes, red chromatophores. The blister on the yolk is the swollen pericardial cavity, and is the only means by which the head end of the embryo may be located.

Fig. 4 represents an embryo of the same age from a weaker solution, viewed from the front. The eyes are represented by the small circles. The semicircular areas, lateral to the eyes, are the distended ear vesicles, whereas the almost circular area reaching from the eyes to the dashed line across the middle of the yolk, represents the distended pericardial cavity. The elongate body, extending from the head ventralward, is the heart. It is beating, but no blood circulates, since no hollow vessels are connected with it. Erythrocytes, represented by stipple, lie in the arterial end of the heart. The lower or venous end of the heart is covered with red chromatophores.

In this same embryo three days later the eyes are degenerating, the left eye being represented by merely a thin smear of retinal pigment

(Fig. 5). The number of chromatophores on the ventral side of the pericardium and on the heart is greater than in a normal embryo. Owing to the swelling of the pericardial cavity, the heart is greatly elongated and its middle portion is transformed into a solid cord.

Sometimes but one eye completely degenerates, resulting in a condition which I have called secondary monophthalmia asymmetrica;

FIGURE 8.



FIGURE 9.

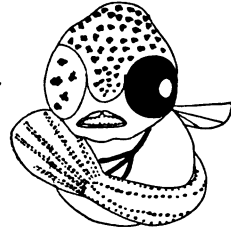


FIGURE 8. — The same embryo as Fig. 7, sixteen days old.

FIGURE 9. — *Monstrum monophthalmicum asymmetricum*, sixteen days old, from M_{800} nicotine solution.

or the two eyes partially degenerate and then fuse together, secondary cyclopia.

Often the anterior region of the head disintegrates, and the cells wander away and become scattered over the pericardium. Such an embryo is shown in Fig. 6, and is atypical only in the fate of the eyes. The heart formed, but did not beat. The stippled area in the tail region represents a mass of erythrocytes. The pericardium was greatly distended and pressed against the ventral side of the head, to which it adhered. The anterior portion of the head disintegrated, and the eyes contracted into two spherical masses blackened by retinal pigment. By the eleventh day the eyes had fallen through the pericardial cavity and become grafted on to the heart.

Only one case of primary cyclopia occurred. This embryo, when four days old (Fig. 7), was apparently normal except for the single dumbbell-shaped eye on the ventral side of the head. The same embryo, sixteen days old, is shown from the front in Fig. 8. Although it had been removed from the nicotine solution when thirty-six hours old and had remained in pure sea water, frequently changed, for two weeks, defects began to appear in the circulatory system, and the circulation ceased entirely long before death. As shown in Fig. 8,

the pericardial cavity is distended and its contents turbid, and include a mass of erythrocytes around the venous end of the heart, represented by stipple.

The only case of primary monophthalmia obtained is shown at the age of sixteen days in Fig. 9. Except for the lack of the right eye, it is apparently normal. Even the right eye socket has developed in an apparently normal manner, but is covered by skin containing chromatophores.

TABLE I

Caffeine . . .	50	80-200	Quinine . . .	200	400
Atropine . . .	90	100	Strychnine	100-1600 (Saturated)
Brucine..	400 (Saturated)
Nicotine . . .	350	400-700	Stovaine . . .	800	1600

The alkaloids, even in very weak solutions, retard development. The toxic limits, and concentrations of solutions causing the abnormalities described above, are given in Table I. A saturated solution of theobromine produced no monsters, due to its very slight solubility, but, from comparative studies on the egg of the sea urchin, we may class it with caffeine.

To give a uniform basis for comparison, the data of only those experiments in which the embryos remained in the solutions thirty-six hours, beginning with the two-cell stage, are used. The "toxic limit" is the solution in which nearly all of the eggs are dead at the end of thirty-six hours. Each number in the table is the denominator of a fraction (of a molecular solution) whose numerator is 1. The middle column gives the toxic limits, and the last column gives the concentrations which suppress the circulation in various degrees, and cause the other abnormalities described above.

Comparative experiments were made on the eggs of the sea urchin, *Arbacia punctulata*. These eggs are more easily obtained, develop more rapidly, and are more sensitive to changes in the medium than are *Fundulus* eggs. All of the alkaloids, as well as stovaine and digitalin, produced the same abnormalities.

The strengths of the solutions are shown in Table II. The eggs were not removed from the solutions and returned to pure sea water, as in the case of the *Fundulus* eggs. The solutions of digitalin are

percentages, in case of the others the number represents the denominator of a fraction (of a molecular solution) whose numerator is 1.

In stronger solutions than those listed in the table, embryos died in segmentation stages. Even in those in the table development was enormously retarded, and the yolk granules dissolved more slowly, although ciliary activity did not appear to be reduced. Whereas

TABLE II.

Caffeine . . .	500	800	Nicotine . .	3,200	6,400
Theobromine	Saturated	Quinine . .	50,000	55,000
Atropine . . .	3,200	25,600	Strychnine . .	25,600	51,200
Brucine . . .	12,800	51,200
Cocaine . . .	6,400	25,600	Stovaine . .	12,800	102,400
Morphine . .	800	6,400	Digitalin . .	.0004%	.0001%

normal embryos, if not fed, starve to death in a few days, some embryos in the alkaloid solutions live several weeks. After the first few days a distention of the body cavity commences and may continue until the ectoderm becomes a very thin-walled vesicle.

In the solutions listed in the middle column plutei were not produced, although rudiments of the skeleton, in the form of tri-radiate stars, sometimes appeared. Some exogastrulæ were produced. In case normal invagination of the endoderm did take place, the gut was never normally differentiated, but usually degenerated into a solid mass of cells.

In the solutions listed in the last column plutei were formed, but there was an early disarrangement of the mesoderm cells, so that the resulting skeleton was abnormal. Since there was an enormous number of forms of the skeleton, they cannot be described here.

CONCLUSIONS.

It has been shown above that the very different organic compounds used, belonging to both the aliphatic and the carbocyclic series, although included in the old class of alkaloids, have the same morphological effects on the eggs of *Fundulus heteroclitus*. Loeb has obtained the same abnormalities in solutions of potassium cyanide and has reared similar monsters from eggs fertilized with foreign sperm.

It might be supposed that these effects follow any injury to the *Fundulus* egg. The distention of the pericardium follows the application of various salts. I have produced it in frog's embryos by mechanical injury. The distention of other serous cavities sometimes follows the application of certain salts and anæsthetics. Loeb prevented the heart beat with potassium salts. Some embryos in ammonium salts were observed by Stockard never to develop a heart beat, and in some in magnesium salts the circulatory system degenerated.

It may be possible that the same abnormalities can be produced by any chemical treatment. However, the quantitative data show striking differences in the effects of different substances. Using solutions of equal toxicity, almost 100 per cent of embryos in ethyl alcohol may show primary defects in the eyes, whereas such defects are seen in not more than one tenth of 1 per cent of embryos treated with alkaloids. On the other hand, the abnormalities described in the circulation may occur in nearly 100 per cent of embryos treated with alkaloids.

Thousands of eggs of *Fundulus heteroclitus* were placed in solutions of each of the alkaloids enumerated, and the eyes of each embryo were carefully examined, but only one case of primary cyclopia and one of primary monophthalmia asymmetrica were observed. These two occurred in the same batch of eggs treated with nicotine. I subsequently used this same concentration of nicotine on the eggs of many different females without reproducing these abnormalities.

Stockard obtained very different percentages of cyclopia in eggs treated with magnesium salts during different seasons or parts of seasons. He once thought this due to accidental variation in the concentration of the solutions. This could not have been the case at least in my experiments, as I used a finely graduated series of concentrations extending a great distance on each side of the apparent optimum for producing cyclopia. In numerous experiments covering an entire season, I failed to obtain as high a per cent of cyclopia with magnesium chloride as recorded by Stockard. By careful measurements of specific gravity, I found that the varying results were not due to differences in density of the sea water. The water I had been using was obtained directly from the sea in glass vessels, but as a control I used sea water drawn from the same pipe as that used by Stockard. This was repeated many times, and it was demonstrated that heavy metals in the water did not account for the differences.

The cause of the varying results must be that different batches of eggs have not the same tendency toward cyclopia, just as the individual eggs laid by the same female at the same time, vary in this respect. Perhaps some *Fundulus* eggs would produce cyclopic embryos without any laboratory treatment, as I have found many cyclopic smelt embryos in the natural breeding places. It would not be safe, therefore, to draw conclusions from two individuals in many thousands. Attention should be directed rather to the quantitative data.

THRESHOLDS OF ELECTRICAL STIMULATION IN THE SPINAL CAT, DETERMINED BY THE MARTIN METHOD.

By E. L. PORTER.

[From the Laboratory of Physiology in the Harvard Medical School.]

INTRODUCTION.

IN April, 1911, Martin¹ published the concluding article of a series of six papers dealing with the calibration of the inductorium. The object of the calibration was to make possible the accurate measurement of the induction shocks used in stimulating irritable tissues, and to state the value of the stimuli in units of such definiteness that they could be duplicated in any properly equipped laboratory. The completion of the calibration is of so recent a date that no considerable amount of data obtained by the method has as yet accumulated. Such a body of data is desirable in order to furnish standards of comparison by which to judge of the irritability of various tissues, under varying conditions, and in the hands of different experimenters. The author has used the method extensively during the last three years, and it is the purpose of this paper to report certain of the results secured, chiefly with the intent of supplying such standards of irritability as have been mentioned above.

METHOD.

The preparation used. — The preparation used has in all cases been the spinal cat.² It has been prepared by a method modified³ from the

¹ MARTIN: this Journal, 1911, xxviii, p. 49. Preceding papers in vols. xxii, xxiv, xxvi, and xxvii. See also MARTIN: The measurement of induction shocks, New York, 1912.

² With exception of the experiment described on p. 144.

³ At the suggestion of Dr. W. B. CANNON.

original one of Sherrington,⁴ as follows: A cat is strapped to the operating board, etherized, a tracheal cannula inserted, and the carotids tied. It is then loosened from the board, turned on its side and a small incision made over the lambdoidal ridge of the skull. A pithing instrument is then thrust through the incision and down alongside the occipital bone until it penetrates the neural canal between skull and atlas. When it strikes the occipital bone on the ventral side of the canal, it is moved from side to side, cutting the cord. It is then thrust forward into the cranial cavity and the entire brain pithed as completely as possible. There is very little bleeding to the exterior. The artificial respiration apparatus, previously set in operation, is now attached to the tracheal cannula. Reflexes can almost instantly be elicited, the scratch reflex often starting spontaneously. Normal body temperature is maintained by an electric heating pad on which the animal lies during the experiment.

The thresholds of stimulation of two different mechanisms have been determined, namely, the flexion reflex of the hind-leg, and a nerve-muscle preparation on the fore-leg, causing extension at the wrist. The reflex is prepared for study as follows: The animal is laid on its side and a slit made in the skin on the side of the hind-leg between heel and knee. The posterior tibial nerve is exposed, freed from the posterior tibial artery and other tissues for 3 or 4 centimetres, and ligated near the calcaneus. It is cut distal to the ligature, and the ligature, followed by the nerve, is drawn into the glass tube and between the platinum contacts of the shielded electrode devised by Sherrington.⁵ To hold the leg firmly in place the skin and muscles of the thigh are cut through and a rigidly supported clamp attached to the femur.

The lower leg and foot are held in loops of leather which are swung by strings from a horizontal bar four feet above the operating table. The whole arrangement is adjusted until the leg is free to flex easily in a horizontal plane. A wire hook is passed through the ankle between the tendo Achillis and the fibula, and a string from this hook passes over a pulley to a muscle lever, writing its record on a drum. If now the tibial nerve is stimulated through the electrodes, the flexion reflex is evoked. In the intact animal this involves flexion at

⁴ SHERRINGTON: *Journal of physiology*, 1909, xxxviii, p. 375.

⁵ *Ibid.*, p. 382.

hip and knee, and dorsi-flexion at ankle. The arrangement described above permits only flexion at the knee to be recorded.

The nerve-muscle preparation causing extension at the wrist is prepared by exposing the dorsal interosseous branch of the radial nerve at the elbow and attaching a Sherrington electrode as in the

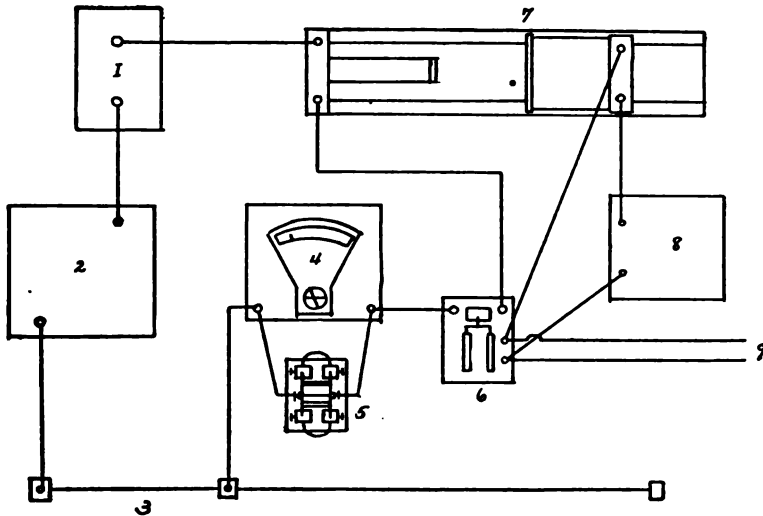


FIGURE 1.—Arrangement of apparatus for the use of the Martin method. 1, battery; 2, resistance box in primary circuit; 3, slide wire resistance for fine adjustment; 4, ammeter; 5, ammeter shunt; 6, make and break key with automatic device for short-circuiting either make or break shocks; 7, inductorium; 8, resistance box in secondary circuit; 9, wires leading to stimulating electrodes.

case of the tibial nerve, but in this case distal to the point of section. The leg is supported by loops of leather, and extension at the wrist recorded by a writing lever, connected to the toes by a string. The movement is of course elicited by stimulation of the dorsal interosseous nerve and is neuromuscular, not reflex.

Procedure in stimulation and in determination of the value of the stimulus.—The results to be reported in this paper will be stated in the “*Z*” units and “*β*” units of Martin.⁶ An example of an actual experiment in which *Z* and *β* were both secured is given below. In this experiment *Z* has been determined under three conditions of secondary resistance for greater accuracy in obtaining *β*. The arrangement of the apparatus is shown in Fig. 1.

⁶ MARTIN: this Journal, 1910, xxvii, pp. 228-230; Measurement of induction shocks, pp. 73-76.

Experiment of Feb. 1, 1912.—The animal, in this case a rabbit, was prepared as described above for the cat. The stimulating electrodes were in contact with the tibial nerve, and were connected with the terminals of the secondary coil through the wires (9). The primary current was made and broken by means of the key (6). With the ammeter shunt (5) set so that 0.1 of the current passed through the ammeter and 0.9 through the shunt, the external resistance in the primary circuit was so adjusted by means of the resistance box (2) and the rheocord (3) that a total current of 0.01 ampere passed through the primary of the inductorium. The key was arranged so that make shocks from the secondary were short-circuited. The secondary was pushed out to the end of the base on which it slides. With the kymograph drum revolving slowly the current was repeatedly made and broken by the key and the secondary gradually moved toward the primary. A point was reached at which there was the slightest perceptible break in the straight line which the recording lever had heretofore been writing on the drum. The threshold of stimulation for the reflex had therefore been reached. The secondary was at 12.8 on the centimetre scale of the inductorium. A calibration table of values of $\frac{M}{L}$ accompanying the inductorium gave 1032 for this position of the secondary. To find Z this number was multiplied by I , the primary current in amperes, according to the formula $Z = \frac{M}{L} \times I$. Z had the value 10.32 (*i. e.*, 1032×0.01). Plugs were now withdrawn from the resistance box (8), throwing 10,000 ohms of additional resistance into the secondary circuit. The position of the secondary at which a break shock just caused reflex contraction was again determined, the value of $\frac{M}{L}$ for this position sought in the table, and Z again calculated. It was now 15.0. The process was repeated with 20,000 ohms in the secondary circuit, giving a third value for Z of 21.36.

The determination of the tissue resistance was next made by the Kohlrausch method.⁷ It was 13,500 ohms. The resistance of the secondary coil, previously determined, was 850 ohms. This gave a total secondary resistance of 14,350 ohms. The last two places are not significant, hence 14,400 was the value used. The data thus far secured are shown in Table I.

⁷ MARTIN: this Journal, 1910, xxvii, p. 228; Measurement of induction shocks, p. 72.

TABLE I.

DATA NECESSARY FOR DETERMINING THE STRENGTH OF A GIVEN STIMULUS BY THE MARTIN METHOD. EXP. OF FEB. 1, 1912.

Resistance of secondary coil	850	850	850
Resistance of tissue	13,500	13,500	13,500
Additional resistance in secondary circuit	0	10,000	20,000
Total secondary resistance (R)	14,400	24,400	34,400
Primary current in amperes01	.01	.01
Position of secondary coil	12.8	11.9	10.9
$\frac{M}{L}$	1032	1500	2136
Z	10.3	15.0	21.4

We thus have three values of Z at three different secondary resistances. These are shown plotted in Fig. 2, with secondary resistances as abscissæ and values of Z as ordinates.

These values form approximately a straight line which cuts the line of zero resistance at about 2.7. This may be considered the value of Z for zero resistance. It is the β of this experiment. To obtain it more accurately than by the graphic method of Fig. 2, recourse was had to two equations,⁸ the first of which is for the constant A . The equation is

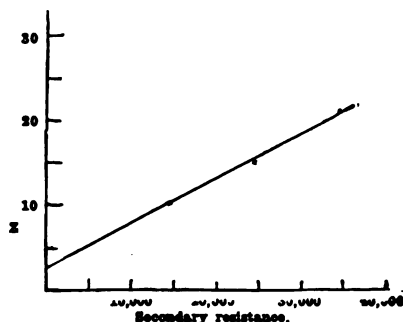


FIGURE 2.—Values of Z of Table I plotted against the resistances at which they were secured.

$$A = \frac{Z_R R' - Z_{R'} R}{Z_{R'} - Z_R}$$

The values for use in this equation are obtained from Table I and are as follows:

R 14,400 ohms	Z_R 10.3
R' 24,400 ohms	$Z_{R'}$ 15.0
R'' 34,400 ohms	$Z_{R''}$ 21.4

⁸ MARTIN: this Journal, 1910, xxvii, pp. 230 and 231; Measurement of induction shocks, pp. 74 and 77.

Substituting and solving with the first and second values of Z and R , we have

$$A = 7500$$

and with the first and third,

$$A = 4200$$

The average of the two values is 5900.

β is obtained from the formula

$$\beta = \frac{A Z_R}{R + A}$$

Substituting and solving, $\beta = 3.0$.

Solving also for Z_R , and $Z_{R''}$, we obtain values of 2.9 and 3.1. The average value from the three determinations is 3.0. The threshold of the flexion reflex in this experiment was therefore 3.0 β units.

RESULTS.

The results to be reported in this paper are the threshold stimuli in Z units and in β units for the flexion reflex and for the nerve-muscle preparation in spinal cats, prepared as above described. They are shown in Table II. The determinations have been made as early as possible after placing the cat on the operating board. The time which elapsed between the etherization of the animal and the determinations is shown in the second column. In most instances the reading was made before an hour and a half had elapsed. Four values secured after longer intervals are included, namely, those at 1.37, 2.09, 2.10, and 3.25, but they show no significant difference from the other values.

The most important results are those of β for the reflex and for the nerve-muscle preparation. The seventeen values for the reflex give an average β of 2.7, and the fourteen for the nerve muscle an average of 1.4. The higher threshold for the reflex is in accordance with other well-known characteristics of reflex arc conduction.*

Values of β for other tissues will shortly be reported by Martin.

* SHERRINGTON: The integrative action of the nervous system, New York, 1906, p. 14.

He has already published ¹⁰ the results of eighteen observations on frogs' gastrocnemii stimulated directly, which gave an average β of 8.1.

In many experimental procedures the calculation of the different values of β is too long a process to be feasible, and the results stated in the Z units are all that the experiment demands. In columns 3 and 6 of Table II are shown threshold values for reflex and nerve-muscle preparation in the Z units. The reflex for sixty-six cases gives an average of 5.2; and the nerve muscle (fifty-two cases) an average of 2.3.

It is of practical importance when results are stated in Z units to know the average ratio of β to Z . Martin finds in the case of the frog tissues referred to above an average ratio of 0.49, and the greatest deviation from this average 0.32, or 35 per cent. The average deviation was 15 per cent. In another series of experiments ¹¹ on frogs' gastrocnemii stimulated through the sciatic he finds an average ratio of 0.48, the widest variation from this average a ratio of 0.28, a difference of 42 per cent. The average variation in this case was 20 per cent. The figures of Table II enable an extension of this calculation to be made. The ratios are shown in columns 5 and 8. The average ratio calculated from the total number in both columns is 0.57. The greatest deviation is with the value 0.13 (Aug. 17, 1910), a difference of 0.44 from the average, or 77 per cent. Other wide variations are those under the dates of July 19, 1910, Aug. 26, 1910, and Sept. 29, 1911. The average deviation is 0.14, or 24 per cent. Thus the author's results show wider variations from the average than Martin's, and a somewhat greater average deviation. The average ratio itself, however, is reasonably close to the two reported by him, differing from them by about 16 per cent.

SUMMARY.

1. A method is described by which the threshold stimuli for the flexion reflex and for a nerve-muscle preparation in the spinal cat have been determined, using the Martin method of measuring the strength of the stimuli.

¹⁰ MARTIN: Measurement of induction shocks, p. 86.

¹¹ MARTIN: *Loc. cit.*

TABLE II.

THRESHOLDS OF STIMULATION FOR THE FLEXION REFLEX AND THE NERVE-MUSCLE PREPARATION IN THE SPINAL CAT MEASURED BY THE MARTIN METHOD.

Date. 1909-1912.	Interval between etherization and observation.	Reflex (flexion of hind-leg by stimulation of tibial nerve).			Nerve muscle (extension at wrist by stimulation of radial nerve).		
		Z	β	$\frac{\beta}{Z}$	Z	β	$\frac{\beta}{Z}$
	<i>h. m.</i>						
Dec. 13 ¹	1.05	3.4	2.0
Jan. 10 ²	0.35	2.4
" 15	0.30	2.0
" 18	0.31	7.9
" 22	0.34	3.1
" 26	0.24	4.3
" 27	0.43	0.7
Feb. 11	0.42	2.4
July 9	1.00	1.7
" 11	0.50	3.2
" 13	0.40	1.7	0.7
" 14	3.25	3.3	2.5	0.75	2.6
" 18	1.20	4.1	2.5	0.61	0.7
" 19	0.55	4.1	3.8	0.92
" 22	0.51	6.1	3.1	0.51	2.9
" 26	0.55	4.1	2.1
" 28	0.47	7.5	3.2	1.7	0.53
Aug. 2	1.06	17.5	6.0	4.0	0.67
" 3	0.55	6.8	3.1	2.1	0.67
" 4	0.51	4.1	0.7
" 5	0.51	9.4	3.8	2.2	0.57
" 8	0.49	6.8	2.9	1.8	0.62
" 9	1.05	5.3
" 12	0.59	2.9	2.1
" 15	1.19	9.4	5.8
" 17	0.52	2.8	3.8	0.5	0.13
" 18	1.37	8.2	1.6
" 20	1.25	5.0	3.8
" 22	1.28	6.2	3.3
" 25	1.25	3.8	4.1
" 26	1.31	6.6	2.4	2.0	0.83
Nov. 2	1.23	7.5	3.6
" 8	1.00	1.6	2.9
Nov. 14	1.08	4.0	1.6
" 19	1.23	4.8	3.5
Dec. 6	1.15	2.4	5.3

2. The average threshold stimulus in β units for the flexion reflex in the spinal cat (seventeen determinations) is 2.7; and for the nerve-muscle preparation (fourteen determinations) is 1.4.

3. The average threshold stimulus in Z units for the flexion reflex

TABLE II—*continued.*

THRESHOLDS OF STIMULATION FOR THE FLEXION REFLEX AND THE NERVE-MUSCLE PREPARATION IN THE SPINAL CAT MEASURED BY THE MARTIN METHOD.

Date. 1909-1912.	Interval between etherization and observation.	Reflex (flexion of hind-leg by stimulation of tibial nerve).			Nerve muscle (extension at wrist by stimulation of radial nerve).		
		Z	β	$\frac{\beta}{Z}$	Z	β	Z
Sept. 7 ¹	h. m.	4.3
" 11	1.23	5.2	0.7
" 14	0.47	8.6	2.9	0.33	2.6
" 16	2.09	3.8	4.9
" 20	0.45	2.7	1.9	0.70	4.5
" 23	2.10	2.6	0.9	0.34	1.2
" 29	1.18	2.3	1.8	0.78	1.1
Oct. 10	1.15	5.3	3.6	0.68	2.7
" 11	1.11	4.5	1.6
" 13	0.57	4.1	1.6
" 17	1.30	3.4	2.0	0.58	0.6
" 18	1.03	7.2	2.4
" 19	0.50	10.5	2.2
" 21	0.49	5.9	1.1
" 24	0.52	2.7	0.5
" 26	0.37	5.5	0.6
" 30	0.43	3.4	2.0	0.58	2.2	1.2	0.54
Nov. 1	1.00	3.2	1.9	0.59	0.7	0.4	0.57
" 2	1.08	21.6	3.5
" 4	0.45	4.8	1.2	0.25	0.9	0.6	0.67
" 14	0.40	2.7
" 16	0.33	3.2	2.4	0.75	2.4	0.5	0.20
" 27	0.40	5.6	0.6
" 29	0.55	7.5	0.7
Dec. 1	0.36	13.7	6.4	0.46	1.1	0.8	0.72
" 5	0.40	2.7	0.5
" 7	0.35	6.1	0.8
" 14	1.04	2.2
Jan. 17 ⁴	0.50	3.6	2.1	0.58	0.8	0.3	0.37
Jan. 30	0.55	7.9	5.4	0.68	4.0	2.0	0.50
Average . .		5.2	2.7	... ⁵	2.3	1.4	... ⁵
¹ 1909. ² 1910. ³ 1911. ⁴ 1912. ⁵ Average for both reflex and nerve-muscle preparation, 0.57.							

in the spinal cat (sixty-six determinations) is 5.2; and for the nerve-muscle preparation (fifty-two determinations) is 2.3.

4. The average ratio of β to Z, calculated from the combined results with the flexion reflex and the nerve-muscle preparation (thirty-

one cases), is 0.57. The average deviation from this ratio is 24 per cent.

I wish to express my thanks to Dr. W. B. Cannon for his helpful supervision during the progress of this investigation, and to Dr. E. G. Martin for assistance in the use of his method.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE
STOMACH. — I. THE CHARACTER OF THE
MOVEMENTS OF THE EMPTY
STOMACH IN MAN.

By A. J. CARLSON.

[From the Hull Physiological Laboratory of the University of Chicago.]

I.

WHILE previous work on parathyroid tetany has focussed the writer's interest on certain phases of the physiology of the digestive tract, it is probable that the present investigation would not have been undertaken except for the fortunate circumstance of securing the services of a young man in normal health with a complete constriction of the œsophagus and an abdominal fistula into the stomach, of sixteen years' standing. The closure of the œsophagus was due to an accident, not to disease. The fistula is three fourths of an inch in diameter, thus allowing ample access to the stomach for various experimental procedures. As nothing pathological could be detected in the man, his digestion being very good and his health normal, he appeared to the writer to be a second Alexis St. Martin, with modifications suited for certain investigations. It seemed that the man should be made use of in the interest of physiology.

The work was begun in May of this year, and some of the phases of it are still in progress. It was found necessary to extend it to other mammals and to birds, for the solutions of certain questions which cannot be worked out on man.

The following points have been established:

1. The empty stomach exhibits, at least during the first twenty-four hours after a meal, two types of rhythmical movements: one is relatively feeble but continuous, with a constant rate of contraction of twenty seconds' duration; the other falls into periods of relatively strong contractions that may end in tetanus. The latter rhythm

involves the fundus. It is not yet determined whether the feebler rhythm is confined to the antrum or also involves the fundus. These movements of the empty stomach are greatly diminished or absent altogether when the health and vigor are in any way impaired.

2. The individual contractions of the group rhythm and the stronger contractions of the continuous rhythm are recognized as hunger pains (confirmation of Cannon and Washburn). There is a close correspondence between the intensity of the hunger experienced by the man and the amplitude of the contractions of the empty stomach simultaneously registered. There may, however, be considerable motor activity of the empty stomach without any definite hunger sensations. The stomach contractions give rise to the sensation of hunger by the stimulation of afferent nerve fibres in the muscle layers.

3. During the strong contractions of the empty stomach the knee jerk is greatly augmented. This seems to indicate an increased tonus of the central nervous system concomitant with, or as a result of, the sensation of hunger.

4. There is an augmentation of the pulse rate during periods of the strong motor activity of the empty stomach.

5. Plethysmograph records of the volume of the arm taken simultaneously with the records of the stomach movements show relatively great fluctuations in the tone of the vasomotor centre during the strong stomach contractions. The types of vasomotor changes seem to indicate a close association between the vasomotor centre and the bulbar tonus centre for the stomach. This instability of the vasomotor tone during the strong stomach contractions may explain the feeling of faintness experienced in strong hunger by many persons.

6. The contractions of the empty stomach are inhibited from the oral cavity: (a) by stimulation of the gustatory nerve endings in the mouth (sweet, bitter, salt, acid); (b) by chewing agreeable, disagreeable, or indifferent substances; (c) by chewing palatable foods when hungry; (d) by smoking; (e) by swallowing movements. The inhibition of the strong "hunger contractions" of the empty stomach by chewing palatable food is prompt, but temporary; hence it appears sooner, but does not last as long as the secretion of gastric juice following this stimulation. The gustatory stimuli that lead to "psychic" secretion of gastric juice thus lead to "psychic" inhibition of the gastric tonus and movements. This fact has also been demonstrated for the dog.

7. The sight or smell of food when in hunger, or any kind of olfactory stimulation, does not appear to affect the stomach movements in the man, but in the dog these stimuli cause inhibition.

8. When the strong contractions of the empty stomach are in progress and in consequence strong hunger sensations are being experienced, V. claims that seeing or smelling palatable foods makes him experience stronger hunger. Since this has no counterpart in increased stomach contractions, it must be due to changes in the brain (confusion of hunger with appetite, or a "facilitation" phenomenon).

9. The rhythms of the empty stomach make their appearance whether the stomach mucosa is acid or alkaline in reaction, but strong acidity or alkalinity causes inhibition. 0.5 per cent HCl causes greater inhibition than a similar concentration of Na_2CO_3 . There is a slow secretion of gastric juice during the contractions; but a rapid secretion of gastric juice is accompanied by depression of the contractions.

10. Some air or carbon dioxide in the stomach cavity does not seem to materially influence the stomach rhythms.

11. The stomach contractions (and the hunger sensations) are not influenced by the introduction in therapeutic quantities into the stomach cavity of phenol, chloreton, orthoform (new), quinine-urea-hydrochloride, or of adrenalin chloride.

12. Water (cold or warm), coffee, tea, beer, wine, and brandy introduced into the empty stomach cause inhibition of the contractions. If introduced suddenly, they may produce one or two immediate contractions, followed by inhibition of varying duration. Of these substances water appears to have the least inhibitory action. There seems to be no increased strength of the contractions following the inhibition periods.

13. During the contraction period bile frequently enters the gastric cavity. In about 50 per cent of the observations bile is found mixed with the small amount of gastric juice always present in the stomach in the morning (6-8 A. M.), that is, twelve to fourteen hours after the dinner. The tests for pancreatic juice have always been negative.

14. During the strong contractions of the empty stomach the secretion of mucin is increased, and there is a decrease in the free HCl, but the secretion is rich in pepsin. In no instance have I found the

stomach free from gastric juice, that is, a fluid containing pepsin, hydrochloric acid, and mucin, in varying concentrations. There are periods of "spontaneous" secretion of gastric juice in the empty stomach, the acidity of which is nearly equal to that of the "psychic" secretion. The gastric juice secreted in consequence of tasting or chewing palatable foods (when hungry) shows a uniform total acidity of 0.50-0.54 per cent (free acid: 0.43-0.48 per cent; combined: 0.03-0.05 per cent).

15. The blood supply of the mucosa of the empty stomach in relative quiescence is such as to give the mucosa a reddish tint equal to the 15 per cent on Tallquist hemoglobin scale. When strong contractions are present, the mucosa appears to become slightly more vascular, the tint being equal to about 30 on the Tallquist scale. It is not clear whether this is due to vasodilation or to mechanical obstruction of the venous outflow. The comparisons are made somewhat uncertain by the rapid movements of the rugæ and the increased secretion of mucin during the contractions.

II.

The man, Fred Vlcek, a native of Trebone, Bohemia, is twenty-seven years old. For the last sixteen years, or since 1897, he has fed himself through a permanent gastric fistula owing to complete closure of the œsophagus, as a result of accidentally drinking a strong solution of caustic soda. I quote the following extracts from a transcript of his case in the records of the Surgical Clinic of the (Bohemian) University of Prague. The transcript is signed by Professor Kukula.¹

In 1891 the boy, Fred Vlcek, then six years old, drank a solution of KOH. A doctor was immediately summoned and acetic acid was administered. The boy was confined to bed for six weeks and lost weight constantly, because he could not swallow on account of the great pain. The parents brought him to Prague, and he was accepted in the clinic of Professor Weiss. He was fed by stomach tube and improved rapidly. The feeding by stomach tube was continued in his home for about a year, when he again was taken to the University clinic in Prague. The œsophagus was so constricted that stomach tube No. 5 could be passed down for a distance of

¹ The translation into English was made by Mr. F. Psota.

14 cm. only. He remained in the hospital from May 17 to June 14. When discharged from the hospital, he weighed 17 K. The feeding per stomach tube was continued in the home, but the constriction of the œsophagus gradually increased. A third period of treatment in the Prague clinic from July 3 to August 24, 1893, resulted in considerable improvement, and when discharged the boy could swallow liquid food fairly easily and without the aid of the stomach tube. The improvement continued so that later he could swallow solid foods, if he pressed on the left side of his throat with his fingers and swallowed with force. In 1896, without any known cause, periodic trouble with the swallowing appeared. These periodic attacks lasted for three or four days and gradually increased in frequency. In consequence he returned to Prague and was received in the surgical clinic of Dr. Maydl, February 5, 1897. A gastrostomy operation was completed February 12, and, being fed through the fistula, the boy gained 7.5 K. in weight up to May 17, 1897. Persistent attempts were made to keep the œsophagus open and to dilate it gradually by sounds, but by May 31 it had closed completely, and steps were taken for an operation on the throat. But the boy, hearing this, escaped from the hospital in a covered coal wagon the following day and was not returned.

Previous to the swallowing of the potassium hydroxide the boy had always been healthy. Since the completion of the gastric fistula in 1897, he has enjoyed good health, except an attack of pneumonia in 1908. Mr. Vlcek came to America in 1910.

Present condition. — Mr. Vlcek is in good physical condition. Height, 5 ft., 8 in.; weight, 62 K. Previous to entering our service he followed the trades of waiter and barber. Despite good average muscular development he is unable to do hard manual labor, such as lifting, etc. He also tends to become nervous on prolonged walking or standing up. With the exception of the closed œsophagus and the gastrostomy, the man is in every respect in normal health, and of good average physical development.

The position of the gastric fistula is shown in Fig. 1. The opening is 8 cm. above the umbilicus and a little to the left of the median line. The opening into the stomach is on the lesser curvature about 4 cm. on the fundus side of the "transverse band," or beginning of antrum pylori. This fact is of importance in interpreting the records obtained by introducing rubber balloons through the fistula. The fistula is large enough to admit a rubber tube half an inch in diameter, and a rubber tube of this size is always kept in the fistula. It is usually

pushed in to a depth of 5 inches. The outer end (about 3 inches) is kept corked and bent at an angle of 45° under the bandage between meals. The tube is changed once a month. Although the tube fits

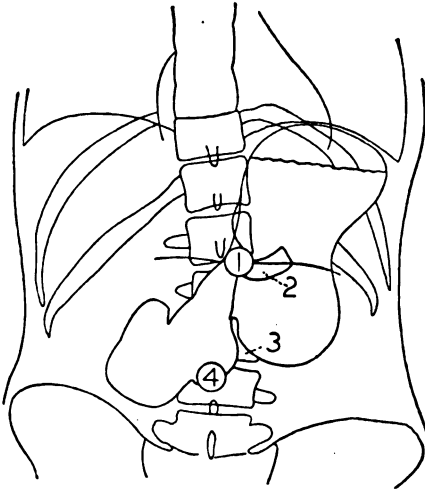


FIGURE 1.—Outline of stomach of V. showing position of the gastrostomy, from an X-ray photograph taken after introducing into the stomach one quart of buttermilk mixed with subnitrate of bismuth. A lead wire was bent around the rubber tube close to the abdominal wall, and the ends of the wires fixed in the form of an X as aid in the orientation. Photograph and tracing by Dr. Hollis E. Potter.¹ 1, external opening; 2, external tube; 3, defect; 4, umbilicus.

rather snugly in the opening, there is some leakage of gastric juice around it, and in consequence some corrosion of the skin for a considerable area about the tube. Since he entered the University service this condition has been greatly improved by more frequent change of dressing and by keeping the skin well covered with vaseline. The stomach mucosa joining the skin or scar tissue has the same appearance and seems to show the same sensitiveness as the rest of the stomach mucosa. But the skin adjacent to the scar tissue is always somewhat hemorrhagic and excessively sensitive to pain, probably due to the corrosion by the gastric juice. The digestive action is, however, counterbalanced by cell proliferation, so that there is no actual lesion.

X-ray photographs and direct inspection by the fluoroscope show that the œsophagus is completely closed at the level of the sternum. There is a slight enlargement or pouch in the œsophagus at that point. He can swallow and hold fluids up to the quantity of 25–30 c.c. Solids of corresponding amounts can be swallowed and held. The evacua-

¹ Dr. Potter makes the following statement: "The size, form, and position of the stomach is that of the orthotonic type. The artificial opening appears to lead to the anterior stomach wall at a point near the centre of the lesser curvature. On the greater curvature at a point almost opposite the artificial opening is a marked defect ('Füllungsdefekt') which is undoubtedly due to contraction of scar following operation."

tion or regurgitation of the œsophageal contents is such an easy performance that I thought at first there must have been established a new co-ordination in way of voluntary control of the vagi-œsophageal neuro-muscular apparatus, and possibly an actual antidromic contraction wave in the functional part of the œsophagus. But further studies showed that the process is purely a mechanical one. He makes an apparently normal swallowing movement, and then a quick bending of the head forward, pressing the chin on the throat, thus forcing the œsophageal contents into the mouth. The usual routine of taking food and the kinds of food taken are as follows: For breakfast, bread or biscuits, soaked and macerated in coffee. The semi-fluid mass is put into a syringe and introduced slowly into the stomach. For lunch, bread, crackers, or biscuit, soaked and macerated in soup or milk, introduced directly into the stomach by syringe. *Very rarely does he take food into the mouth at breakfast or lunch.* The evening meal consists of soup, meat and potatoes, vegetables, etc., and coffee or milk. The food is put in the mouth, thoroughly masticated, and then introduced into the stomach by syringe; or a rubber tube long enough to reach the mouth is connected with the fistula tube, the other end of the tube placed in the mouth, and the masticated food blown into the stomach. The syringe method is usually followed. A glass of water is always introduced into the stomach before going to sleep at night. He does not use tobacco in any form, but takes beer and wines occasionally.

III. EXPERIMENTAL PROCEDURES.

1. **Stomach contractions.** — The stomach contractions were recorded by means of a balloon in the stomach connected (air transmission) with a water or bromoform manometer. The balloons used varied in capacity from 75 to 150 c.cm. When bromoform manometers were used a manometer tube 1.5 cm. in diameter was found serviceable. The air pressure in the balloon with the stomach at relative rest was usually adjusted at 3–6 cm. of bromoform. After trying out balloons of varying capacities and thickness, I concluded that a condom of the thinnest rubber available in the market was the most serviceable, and this was used in most of the work. The advantage of such a delicate balloon is in recording the weaker tonus changes or contrac-

tions, and the pulse beat in the stomach. The only disadvantage is the occasional breaking of the condom by an exceptionally sudden and vigorous stomach contraction, especially when a bromoform manometer is used for recording.

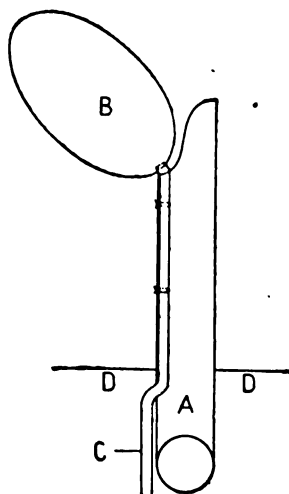


FIGURE 2.— Diagram illustrating the arrangement of the balloon and manometer connection for the purpose of recording the stomach contractions simultaneously with other experimental procedures in the gastric cavity. *A*, fistula tube (normal); *B*, balloon; *C*, tube connecting balloon with the manometer; *D*, *D*, line of abdominal wall.

In the experiments simply involving the recording of the stomach contractions under varying external conditions, the balloon was introduced through the permanent rubber tube in the fistula. The tube connecting the balloon with the manometer was passed through the cork fitting into the fistula tube to prevent escape of gastric juice and of entrance of air into the stomach.

2. In the experiments involving examination of the inside of the stomach, recording the secretion of gastric juice, or the introduction of various substances into the stomach simultaneously with the stomach contractions, the balloon was adjusted in the manner shown in Fig. 2. An opening was made in the wall of the permanent fistula tube 2 centimetres external to the abdominal wall, and the balloon tube passed through this opening and securely fixed to one side of the fistula tube. A small-sized catheter for collecting the gastric juice was similarly fixed to the opposite wall of the fistula tube or simply introduced through an opening in the cork.

When the balloon is fixed to the permanent fistula tube in this manner, gastric juice or contents can be withdrawn from the stomach, the mucosa directly inspected, and foods or liquids introduced in the normal way while the stomach contractions are being recorded.

In most of the experiments *V.* was sitting down in an easy-chair or reclining on a couch. A few observations were taken with the subject standing up or walking.

IV. THE CHARACTER OF THE MOVEMENTS OF THE EMPTY STOMACH.

1. The movements of the empty stomach in mammals were first studied by Boldireff² in dogs by means of the gastric fistula. Rubber balloons were introduced into the stomach and connected by air or water transmission to the recording manometer. According to Boldireff the empty stomach of the dog exhibits alternating periods of rhythmical contractions and periods of complete quiescence during the first three or four days of fasting. The periods of activity vary in length from twenty to thirty minutes, and the intervening periods of rest last from one and a half to two and a half hours. Both the fundus and the pyloric region of the stomach are involved in the activity of the contraction period, the fundus giving 10 to 20 very vigorous contractions. Boldireff states definitely — and the published tracings seem to support the statement — that between the periods of strong rhythmical contractions the stomach is in complete rest.³ The period of activity begins with weak contractions, and these increase gradually in strength until the period ends abruptly with the strongest contractions. Inasmuch as the tracings published by Boldireff do not show the stomach respiratory pressure or the stomach pulse pressure, it would seem that the methods of registration were not delicate enough to detect feeble rhythms of contractions that might have been present during the periods of relative rest.

Cannon and Washburn⁴ studied the movements of the empty stomach in man by introducing a balloon through the œsophagus into the stomach. The observations were made six to twenty hours after meals. They found that the periodic activity of the empty human stomach is very similar to that in the dog, but the average duration of the periods is not given. The fundus contractions were about thirty seconds in duration, and the pause between the contractions lasted about sixty seconds. The published tracings show a gradual tonus contraction of the fundus during the pause. The observations of Cannon and Washburn were directed towards establishing the re-

² BOLDIREFF: Archives des sciences biologiques, 1905, xi, p. 1; Ergebnisse der Physiologie, 1911, xi, p. 120.

³ "Während der Ruheperiode ist der Magen untätig, es tritt in ihm keine einzige, nicht einmal eine kleine Kontraktion ein." *Loc. cit.*, p. 186.

⁴ CANNON and WASHBURN: this Journal, 1911, xxix, p. 441.

lation between the contraction periods of the stomach and the sensation of hunger. They seem to agree with Boldireff in the absolute quiescence of the stomach between the periods of the strong rhythmical contractions. "Before the hunger was experienced by W. the recording apparatus revealed no sign of gastric activity."⁵ There is some indication of a feeble rhythm during the rest period in one of their published tracings (Fig. 2).

2. During the time from May 27 to August 29, 1912, records of the movements of the empty stomach were obtained from V. on sixty different days. The time of observation varied from two hours to twenty-four hours after meals. The bulk of the observations were begun at 10-11 A. M. after a light breakfast at 6 or 6.30 A. M. The breakfast consisted of one, two, or three biscuits soaked and macerated in coffee (without cream).

[When the pressure in the balloon in the stomach is properly adjusted and the manometer-recording devices made as delicate as possible, the tracings obtained form a composite of the following pressure variations in the gastric cavity:

(1) Periods of powerful rhythmical contractions, alternating with periods of relative quiescence. As the duration of each individual contraction in these periods is approximately thirty seconds, we will designate these periods the "thirty-seconds rhythm."

(2) A pressure rhythm (tonus contraction of fundus or peristalsis of the antrum) of wonderful uniformity in rate, the rate varying from eighteen to twenty-two seconds with an average of twenty seconds. This pressure rhythm increases in intensity without change in rate during the periods of the powerful rhythmical contractions of the fundus, and are weakest immediately after these periods. But they are always present in the empty stomach, provided the subject is healthy in every respect. For the sake of brevity we shall designate these contractions provisionally as the "twenty-seconds rhythm."

(3) A pulse pressure rhythm, always present.

(4) A respiratory pressure rhythm, always present.]

3. The periods of relatively powerful rhythmical contractions are practically identical with the periods of "hunger contraction" of Cannon and Washburn, and correspond closely to the contraction periods studied by Boldireff in the dog's stomach. The contractions

⁵ *Loc. cit.*, p. 410.

of these periods usually begin as feeble tonus rhythms; they gradually increase in amplitude *pari passu* with shortening of the intervening pauses, and may or may not end in tetanus or prolonged tonus contractions, followed by a relatively abrupt relaxation and quiescence.

[When the contractions are relatively feeble, the periods of activity are always short, the variation being from six to twenty minutes, with an average duration of twelve minutes. The number of strong contractions in these periods varies from 10 to 25 with an average of about 14 contractions. The duration of each individual contraction is approximately twenty to twenty-five seconds. The stronger contractions are usually in the middle of the periods, the initial and final contractions being the weakest. In no case have I seen such a period end in tetanus. A typical record of one of these contraction periods is given in Fig. 3. When the stomach exhibits these periods of relatively feeble rhythm, the interval between each period varies from five to twenty minutes.]

[The periods of more powerful contractions exhibit some characteristic features. The periods are always initiated by weak contractions with long intervening pauses. These pauses may be of several minutes' duration. Then the individual contractions gradually increase in amplitude, and the intervening pauses become shorter, until the climax is reached in a number of very powerful and rapid contractions approaching complete tetanus. The tetanus usually lasts from two to five minutes. The cessation of these periods of activity is always abrupt. There may be two or three periods of nearly complete tetanus at the end of the period. On five different days these final tetanus periods lasted for from ten to fifteen minutes. This is, however, exceptional. When the period does end in tetanus, the tetanus usually lasts only two to three minutes. A typical tracing of one of these

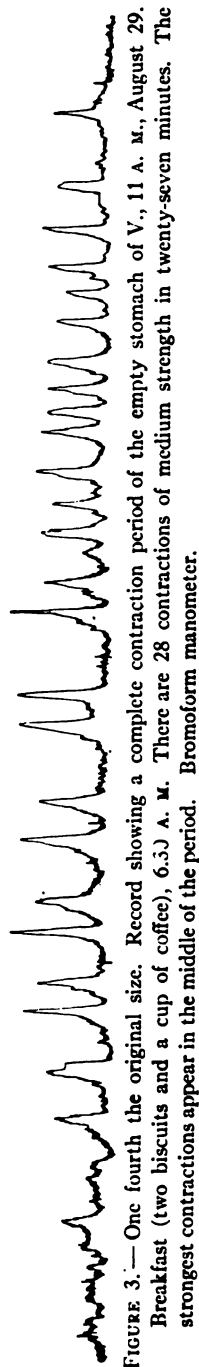


FIGURE 3.—One fourth the original size. Record showing a complete contraction period of the empty stomach of V., 11 A. M., August 29. Breakfast (two biscuits and a cup of coffee), 6.30 A. M. There are 28 contractions of medium strength in twenty-seven minutes. The strongest contractions appear in the middle of the period. Bromoform manometer.

vigorous periods ending abruptly in tetanus is shown in Fig. 4. The duration of each contraction varies from twenty seconds to thirty seconds. The contraction time is shortest at the final stage of greatest activity. When the contrary appears to be the case, the tracings show that the prolonged curve is a fusion of two or more contractions. The interval between the contractions varies from two to five minutes at



FIGURE 4. — One third the original size. Record showing final fourth of a period of strong contractions of the empty stomach, ending in nearly complete tetanus, 11 A. M., August 6. Light breakfast at 6 A. M. Bromoform manometer. A, stomach contractions; B, respiration. Bromoform manometer.

the beginning of a period to nothing at the end. The duration of the period varies from one half to one and one half hours. Thirty to forty-five minutes is the usual run, the longer periods being exceptional, when there is no experimental interference with the stomach. The number of individual contractions in a period varies from 20 to 70.

The characteristic beginning of these strong periods of activity is shown in Fig. 5. This initial stage may be described as a slow tonus rhythm, the contraction phase being very gradual and of two to three minutes' duration. The relaxation phase is always more abrupt. The development of the activity period out of this initial slow tonus rhythm seems to take place mainly in the contraction phase, a gradual increase in the amplitude and in the rapidity of contraction.]

There remains to be noted a rather atypical form of activity of the empty stomach occasionally observed. This consists in contractions, feeble or powerful, that do not fall into distinct groups or periods. These contractions are usually irregular both in strength and in rate. The average rate is slow, the interval between the contractions varying from five to ten minutes. Similar solitary contractions may also appear in the interval between two typical periods of rhythm. These contractions may come two or three in sequence typical of the beginning of an activity period, but instead of gradually increasing activity

the stomach relapses into relative quiescence for another ten to thirty minutes.

In the total of sixty days of observations on which the present discussion is based, the empty stomach of V. failed to show the above

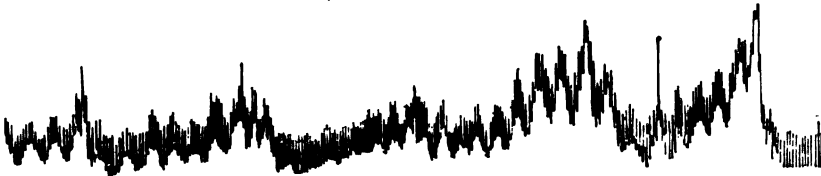


FIGURE 5. — About one third the original size. Record showing the beginning of a period of strong contractions of the empty stomach. The contractions of the twenty-seconds rhythm are superimposed on the slow tonus contractions, which initiate the period. Bromoform manometer.

rhythm periods on six days only. On two of these days there was some disturbance of the alimentary tract (one day diarrhoea, the other day some pain or discomfort in the epigastric region, but no inflammation of the gastric mucosa). On three occasions V. came to the laboratory in the morning after having had a rather restless sleepless night. In



FIGURE 6. — Records showing the increase in strength of the twenty-seconds contractions during the pause between the periodic rhythm. I, five minutes after a strong contraction period (ending in tetanus); II, fifteen minutes later; III, ten minutes later. No. III shows at 4 the very beginning of the next period. A, stomach contractions; B, respiration. Bromoform manometer.

two cases this was evidently due to oppressive heat and humidity. On these mornings V. complained of "not feeling very well," or "feeling a little tired." Even on these days the stomach was not completely quiescent. The rapid or twenty-seconds rhythm could usually be demonstrated, and also some slow feeble and irregular tonus changes, but these tonus contractions failed to develop into the characteristic rhythm periods. There would have been a few more days of absence of the activity periods if observations had been made on all the days when V. "did not feel quite right." But after the uniform failure under

the above conditions, work was done only on days when V. appeared and felt normal, which was almost every day.

[4. Tracings illustrating various phases of the rapid or twenty-seconds rhythm are reproduced in Figs. 6-7. These contractions are weakest immediately after the strong contractions periods, and during the intervening pause between these periods. The contractions in-

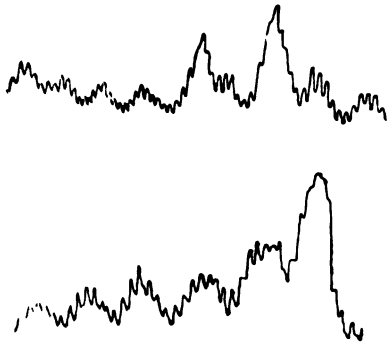


FIGURE 7. Two thirds the original size.

Records showing the increase in strength of the twenty seconds contractions. Occasionally these twenty-seconds contractions approach the strength of the group rhythm. Bromoform manometer.

crease in amplitude somewhat prior to and during the first part of the thirty-seconds rhythm period. During maximum activity in the latter period the former rhythm may be obscured, or not show at all on the tracings, owing obviously to the increased pressure in the balloon, but it is in evidence again on the cessation of the tetanus or strong contractions. The amplitude of contractions in the twenty-seconds rhythm is usually very uniform, except for the gradual increase just noted (Fig. 6). But occasionally the amplitude of one or two of the contractions may

approach those of the thirty-seconds rhythm, as shown in Fig. 7. When this occurs, the contractions are recognized by V. as "hunger contractions" or "hunger pains." The rate of the contractions is very much more constant than the amplitude. Hundreds of these contractions have been measured and the time found to vary between eighteen and twenty-two seconds with the average time of twenty seconds. The reader will recall that this rate is identical with that of the peristalsis of the antrum during normal digestion in man.

The nature of the twenty-seconds rhythm is not yet definitely established. I thought at first that it might be due to a periodicity in the respiratory movements. But this possibility is definitely disproved by the fact that no such rhythm appears on the tracings of the respiratory movements (chest pneumograph). The rhythm also recalled the Traube Hering waves of arterial blood pressure, with a suggestion

of the possibility that the rhythm under discussion is of vasomotor origin, the wall of the stomach (when in relative tonus) acting as a plethysmograph. If this is the case, the increased amplitude of the "contractions" during the thirty-seconds rhythm would indicate a greater blood flow through the stomach and possibly a greater instability of the vasomotor mechanisms during these periods. The full discussion of these points is deferred to a later paper, which will deal with the relation of the tonus mechanism of the stomach to the vasomotor mechanism.

While it is a fact that the stomach pulse of the empty stomach, which may be seen by direct inspection by the aid of a small electric lamp in the stomach cavity, appears on the tracings (see Fig. 8), and therefore the possibility of the vasomotor origin of the twenty-seconds rhythm not entirely excluded, yet the striking uniformity in the rate and the identity of the rate with that of the antrum peristalsis in digestion argue against this origin. At least I know of no vasomotor rhythm of such *uniformity of rate*. This seems to leave two possibilities — the contractions represent the rhythm of the fundamental tonus of the neuro-muscular mechanism of the fundus or body of the stomach, or they represent the peristalsis of the antrum. The following facts seem to argue against the former assumption: (a) The rhythm was not observed by Cannon and Washburn. In their method of observations the balloon in the stomach was fixed in the cardiac end. The absence of the rhythm in the work of Boldireff on dogs is of less significance, because it is quite clear that the method used was not delicate enough to disclose it. (b) So far as I know, no such rhythm has been noted by previous observers on the tonus of

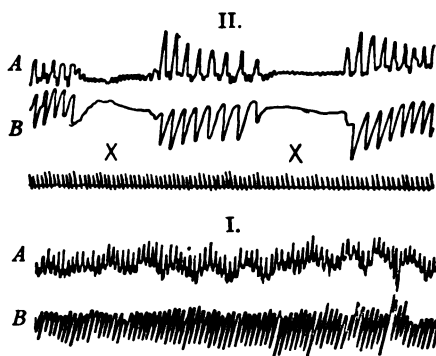


FIGURE 8. — Two thirds the original size. Record showing the pulse beat in the empty stomach. *A*, record from stomach; *B*, respiration. At *x* Mr. V. inhibited the respiratory movements, and as a result the balloon in the stomach shows clearly the pulse beats. No. II was taken only a few minutes after No. I. The twenty-seconds rhythm is not shown clearly in the former, because of the greater rapidity of the recording surface. Time, seconds.

the fundus in mammals, but this may not be so significant in view of the fact that practically all of this work has been done under conditions that in all probability would depress if not disorganize the *normal* tonus mechanism. However, the prevalent view seems to be that, aside from the periodic activity of strong rhythmical contractions, the tonus of the fundus is a more or less persistent contraction without any rhythmical oscillations.

In this connection I desire to call attention to the findings of Stübel ⁶ on the empty muscle stomach of birds (pigeons, chickens, ducks). The intact as well as the isolated muscle stomach in tonus of an apparently constant character shows a rhythm of the action current varying from 50 to 150 per second. Stimulation of the peripheral ends of the vagi usually augments the rate and strength of the rhythm. This rhythm of the action current is, of course, too rapid to be directly compared with our twenty-seconds rhythm in the stomach of man, especially since Mangold ⁷ has shown that the actual contractions of the muscle stomach of birds do not exceed the rate of 3 to 5 per minute. But it suggests that the cause of the tonus changes revealed by relatively crude and violent methods is to be sought in more primary and rapid rhythms of activity, requiring for their detection a closer approximation to the normal condition of the entire animal.

The possibility that the twenty-seconds rhythm is in reality the antrum peristalsis is, of course, strengthened by the fact that the fistula opening into the stomach of V. is not far from the antrum. The side of the balloon towards the antrum may be affected by the antrum contractions, or the balloon, not being anchored to the cardia and œsophagus, may be pushed into the antrum by the fundus contractions. There seems no way of settling the question on V. But I propose to determine the point on dogs by a pouch of the fundus isolated according to the method of Pawlow.

5. The present work reveals nothing new as regards the intragastric pressure rhythm due to the movements of respiration. This respiratory pressure does not appear on the tracings during the gastric tetanus or at the apex of the strong contractions. When the stomach is relatively quiescent, forcible expirations (coughing, sneezing) may produce greater intragastric pressure than the maximum gastric contraction, the water or bromoform being forced out of the manometer.

⁶ STÜBEL: Archiv für die gesammte Physiologie, 1911, cxliii, p. 381.

⁷ MANGOLD: Archiv für die gesammte Physiologie, 1911, cxxxviii, p. 1.

6. **The stomach pulse.** — When the empty stomach is moderately contracted, direct inspection by the aid of a small electric bulb in the gastric cavity shows distinct oscillations of the rugæ synchronous with the arterial pulse. The oscillations of the rugæ cause similar oscillations of the gastric juice (mixed with mucin), which is always present in the otherwise empty stomach. When the strong contractions (thirty-seconds rhythm) appear, the pulse oscillations of the rugæ seem to disappear, either because of the greater rigidity of the stomach folds, or else owing to the difficulty of distinguishing the pulse oscillations when the rugæ are closely packed and slide rapidly over and past one another, as they do when the fundus contracts. The picture revealed by the gastric cavity when the stomach is in a period of rhythmic contractions is interesting, but rather bewildering, and I have ceased to wonder how Beaumont could have so completely failed to grasp the character of the stomach movements in digestion.

DISCUSSION OF THE RESULTS.

The shorter periods of relatively feeble contractions described in the previous pages are evidently identical with those reported by Boldireff in the dog and by Cannon and Washburn in man. The only difference appears to be a shorter interval or period of quiescence between each period of activity in the case of the results on V. The periods of stronger rhythm usually ending in tetanus are of much longer duration than those given by Boldireff and by Cannon and Washburn. The tetanus feature, as well as the twenty-seconds rhythm, appears to be a phenomenon not previously noted. It seems likely that there is no essential difference in the two types of the thirty-seconds rhythm, the longer and more powerful period simply indicating a condition of greater excitability and contractility of the stomach. It is, indeed, possible that this degree of activity of the empty stomach may be present in young and vigorous individuals only. It is certainly not present unless the individual is in the best condition.

Our results seem to challenge the correctness of the statements of Boldireff and of Cannon and Washburn, that the stomach is completely at rest between the periods of the thirty-seconds rhythm. According to Mangold,⁸ the completely empty muscle stomach of the buzzard

⁸ MANGOLD: *Archiv für die gesammte Physiologie*, 1911, **ccxxix**, p. 10.

is also quiescent. Rossi⁹ claims, however, that the activity of the stomach of chickens is more vigorous when empty than during digestion. It is clear that the empty stomach of V. is never completely quiescent, at least during the first twenty-four hours after an ordinary evening meal or dinner. For even should the twenty-seconds rhythm be shown to be a pylorus rhythm, this activity of the pylorus could probably not go on without considerable tonus of the fundus musculature.¹⁰ It is not improbable that this persistent motor activity of the empty stomach is present only in vigorous individuals.

The reader may question the accuracy of denoting the contractions described by Boldireff, Cannon and Washburn, and by the writer as motor activities of the *empty* stomach. In all of these cases the stomach was certainly empty of food. That was rendered certain by direct inspection in the present work. But may not the distended balloon act as food in so far as the food acts mechanically in the way of producing stomach movements? This is, indeed, claimed to be the case by Mangold for the muscle stomach of the buzzard. The metal cannula in the case of the dog and the rubber tube in the case of V. may, of course, act in the same direction. Indeed, what answer can we make to the objection that the persistent motor activity of the stomach of V. is due to the mechanical stimulation of the balloon and the ever-present rubber tube, and must therefore be considered as an abnormal condition? It is not difficult to prove that certain forms of mechanical stimulation, such as the sudden distention of a rubber balloon in the gastric cavity, may cause brief contractions in the stomach, but it can be shown just as conclusively, I think, that the stomach rhythms described above are not caused by the presence of the foreign objects in the stomach. This will be made evident in a later report dealing with certain conditions affecting the movements of the empty stomach.

⁹ Rossi: *Archive di fisiologia*, 1905, ii, p. 375.

¹⁰ CANNON: *this Journal*, 1911, xxix, p. 250.

ON THE CREATIN-SPLITTING ENZYME OF THE PARATHYROIDS AND THE ADRENALS.

By ALBERT HOLMES ROWE.

[From the Rudolph Spreckels Physiological Laboratory of the University of California.]

SOME symptoms in parathyroid tetany markedly resemble the effects of creatin, described by Landois¹ and later by Maxwell,² when the creatin is applied to the motor areas of the cerebral cortex. This fact suggested the possibility that the tetany in a parathyroidectomized animal is produced by a nitrogenous substance, like creatin, which normally is changed into a harmless compound by the action of the parathyroid glands.

That tetany may be due to such a metabolic toxin, which is ordinarily destroyed by parathyroid activity, had been previously suggested. Berkeley and Beebe³ lay emphasis on this idea, — that tetany is due to a metabolic poison. They base their opinion on the following facts: the symptoms of tetany seem to have a central origin and those of acute tetany are similar to those due to the poisoning of the central nervous system by strychnine; the symptoms are increased by a meat diet; the symptoms are relieved by bleeding, followed by transfusion, which would rid the body of any circulating metabolic poison. This hypothesis is antagonistic to the calcium regulating theory of MacCallum and Voegtlin,⁴ placing the nitrogenous substance as the primary cause of the tetany and recognizing any calcium change which occurs as a secondary effect.

In view of the possibility that the symptoms of tetany might be produced by such a substance, it seemed worth while to test out the

¹ LANDOIS: Deutsche medicinische Wochenschrift, 1887, xiii, p. 685.

² MAXWELL: Journal of biological chemistry, 1907, iii, p. 21.

³ BERKELEY and BEEBE: Journal of medical research, 1909, xx, p. 149.

⁴ MACCALLUM and VOEGLIN: Journal of experimental medicine, 1909, xi, p. 118.

action of a parathyroid extract on such a nitrogenous substance, creatin being the most obvious one.

Gottlieb and Stangassinger⁵ have investigated the action of the extracts of various organs and tissues of the body on creatin and creatinin. They found that extracts of the kidney and liver of the ox and of the thyroid gland of the dog break down creatin and creatinin into lower nitrogenous products, the loss varying from 50 per cent to 99 per cent. But these experiments lasted from forty-eight hours to many days, and it was hoped that we should find an extract which decomposes creatin in a short time, such as must take place in the body itself.

It was found impossible to procure parathyroid glands in sufficient amounts to carry out a satisfactory test of the effect of their extract on creatin. But since the thyroid glands of the sheep were obtainable and since it has been pointed out by Forsyth⁶ that parathyroid tissue is distributed throughout the thyroids of the sheep, an extract of these glands was tested out, with the hope that any pronounced action of the parathyroids would thus be shown by an increased rapidity in the breaking down of creatin.

METHOD.

Immediately after the death of the sheep at the slaughter house, the windpipe was excised, one cut being made above the larynx and another at about the eighth to the tenth ring of the trachea. The thyroid apparatus was then dissected out and placed in a bottle containing a little toluol, and kept at freezing temperature from two to four hours until ready for use.

An emulsion of the active agent of the glands was prepared at first according to the method described by W. Wiechowski.⁷ But because of the length of this method, it was replaced by that of Gottlieb and Stangassinger,⁵ which was followed throughout the experiments.

The gland was crushed in a meat chopper and then ground with sand and a little M/6 NaCl in a mortar for at least half an hour.

⁵ GOTTLIEB and STANGASSINGER: *Zeitschrift für physiologische Chemie*, 1907, v, p. 52.

⁶ FORSYTH: *Journal of anatomy and physiology*, 1907-1908, xlii, p. 142.

⁷ WIECHOWSKI, W.: *Beiträge zur chemischen Physiologie*, 1906-1907, ix.

A larger amount of M/6 NaCl was then added, and the resulting mixture was strained through cloth. The turbid, opalescent emulsion was then added to the flasks containing the creatin solution. Ten cubic centimetres of this ferment emulsion were placed in a clean flask with 10 c. c. of a .5 per cent solution of creatin, and from 1½ to 2 c. c. of toluol were added as a preservative. The required number of flasks were filled and were then incubated at 37° C. for a period varying from eighteen to seventy-two hours. The contents of each flask were then diluted with about 150 c. c. of a 5 per cent boiling solution of NaCl. Dilute acetic acid was then added so as to make the solution just acid, and the whole was quickly boiled. The proteins were thus precipitated, and filtering gave a clear fluid which would not interfere with the color reaction. This solution was neutralized, then, by adding barium carbonate, was filtered, and evaporated to about 80-100 c. c.

In order, now, to determine the amount of creatin plus creatinin left in the flask undecomposed, the contents of the flask were autoclaved with 10 c. c. N/1 HCl for twenty minutes at 20 pounds' pressure. By this method it has been found by Benedict and Meyers⁸ that all of the creatin is changed into creatinin. The determination of the resulting creatinin was done by Folin's⁹ colorimetric method.

RESULTS.

That creatin does not break down in appreciable amounts when left standing in a watery solution to which toluol has been added was shown by Gottlieb and Stangassinger.¹⁰ Their tabulated results show a loss of only 0.58 per cent to 1.61 per cent in from one to six days. Thus it is safe to assume that any large decomposition of creatin observed in our experiments was due to the action of the gland extract.

These experiments do not show any great decomposition of creatin. Neither is the velocity of the reaction such as would be expected if this reaction played an important part in the animal metabolism. The results are comparable to those of Gottlieb and Stangassinger, already referred to.

⁸ BENEDICT and MEYERS: this Journal, 1907, xviii, p. 397.

⁹ FOLIN: Zeitschrift für physiologische Chemie, 1904, xli, p. 223.

¹⁰ GOTTLIEB and STANGASSINGER: *Loc. cit.*

EFFECT OF A THYROID EXTRACT OF THE SHEEP ON CREATIN.

Number of experiment.	Length of experiment.	Amount of creatin added.	Amount of creatin lost expressed as creatinin.	Per cent of loss.
	hr.	mg.	mg.	
1	16	50 ¹	13.395	35.30
2	16	50	12.628	33.84
3	16	50	10.940	28.83
4	18	50	17.690	46.62
5	18	50	14.117	37.20
6	48	50	27.815	70.60
¹ Fifty mg. of creatin when changed into creatinin should yield 37.94 mg.				

It was, however, suggested to me by Professor Maxwell that the thyroid gland of the sheep, which contains some parathyroid tissue, might have a ferment which, when activated by some ferment of another gland of internal secretion, would decompose creatin rapidly. This is entirely possible, since such interrelations have been pointed out, as described by Hoskins¹¹ and others. It was thus decided to test the combined action of the sheep's thyroid extract and its adrenal extract on creatin.

A few experiments were first made to determine the effect of adrenal extract when acting alone on creatin.

EFFECT OF ADRENAL EXTRACT ON CREATIN.

Number of experiment.	Length of experiment.	Amount of creatin added.	Amount of creatin lost expressed as creatinin.	Per cent of loss.
	hr.	mg.	mg.	
7	16	50	14.117	37.20
8	16	50	13.395	35.30
9	16	50	14.117	37.20
10	48	50	20.888	55.05
11	72	50	26.340	69.40

¹¹ HOSKINS: American journal of medical science, 1911, cxli, p. 374.

Creatin-Splitting Enzyme of Parathyroids and Adrenals. 173

These results show that the adrenal possesses about the same power to decompose creatin as do the other tissues and glands which have been tested.

The action of the combined extracts of thyroid and adrenal glands was then tested, 5 c. c. of each extract being used, making a total of 10 c. c. of ferment emulsion, which was equal to the amount used in previous experiments.

EFFECT OF COMBINED EXTRACTS OF THYROID AND ADRENAL ON CREATIN.

Number of experiment.	Length of experiment.	Amount of creatin added.	Amount of creatin lost expressed as creatinin.	Per cent of loss.
	hr.	mg.	mg.	
12	16	50	14.117	37.20
13	16	50	12.628	33.84
14	16	50	14.117	37.20
15	16	50	13.395	35.30

These results compare favorably with those obtained when thyroid and adrenal extracts were used alone. It seems, therefore, that neither of these extracts has the power to activate the other.

SUMMARY.

1. The results of Gottlieb and Stangassinger are confirmed as to the presence of a creatin-splitting ferment in the thyro-parathyroid tissues.
2. A similar ferment is found in adrenal extract.
3. There is no evidence that either the parathyroids or the adrenals contain a creatin-splitting ferment which can be activated by the other.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH.
— II. THE RELATION BETWEEN THE CONTRACTIONS
OF THE EMPTY STOMACH AND THE SENSATION OF
HUNGER.¹

By A. J. CARLSON.

[From the Hull Physiological Laboratory of the University of Chicago.]

I.

IN view of the fact that the literature on the nature of the sensation of hunger is exhaustively reviewed in the recent paper by Cannon and Washburn² an extended critique of the facts and theories at this time is superfluous. But the main theories, together with the facts adduced in their support, may be recounted here for the purpose of orientation.

(1) Hunger is a general sensation due to certain conditions of the metabolism in all the tissues, or more particularly in the nerve cells.³— In support of this view have been cited such facts as the uncertainty of the peripheral reference of the sensation; the alleged persistence of hunger after total excision of the stomach or after section of the vagi and high spinal transection; the alleged persistence of hunger when the stomach is filled with food, etc. As regards total excision of the stomach, this is, strictly speaking, impossible, or at least has not yet been made. Some stomach tissue is invariably left below the diaphragm in order to render continuity of the digestive tract possible, and this stomach remnant has a tendency to hypertrophy or dilate into a considerable stomach pouch. Moreover, in the clinical cases it is not clear that the observer has differentiated between *appetite* and *hunger*. In the case of experimental animals the criterion for hunger

¹ The reader is referred to the first article (this Journal, 1912, xxxi, p. 151) for an account of the methods, etc., employed in the experiments reported in this paper.

² CANNON and WASHBURN: this Journal, 1912, xxix, p. 441.

³ This view is elaborated in great detail by TURRO in a series of articles in *Zeitschrift für Psychologie und Physiologie des Sinnesorganes*, 1910-1911, xlv and xlv.

has been the interest in or eagerness for food. This is a criterion for appetite, but does not necessarily involve hunger. The persistence of hunger when the stomach is filled with food should be recognized as a special pathological condition ("polyphagia").

The most cogent objections to the above theory are the practically universal reference of the hunger sensation to the stomach or epigastrium; the intermittency of the sensation, at least in moderate hunger; the temporary abolition of the hunger, in normal persons, by anything, even indigestible materials, introduced into the stomach.

(2) **Hunger is caused by the stimulation of afferent nerves in the stomach by the distention of the tubules or ducts of the gastric glands owing to the accumulation in them of gastric juice.**— This is the "turgescence theory" of Beaumont. It would account for the reference of the hunger sensation to the stomach, but I know of no other fact that can be adduced in its support. Accurate knowledge of the structure of the gastric glands would have rendered the promulgation of the hypothesis impossible. The hypothesis has lived, because linked with the name of Beaumont. The peculiar and sudden pouring out of gastric juice during or after states of hunger described by Beaumont in connection with the statement of his theory of hunger is an error of observation, or pathological. At least I have never observed this phenomenon in V.

(3) **Hunger is due to the stimulation of afferent nerves in the stomach by the contraction of the stomach musculature (Weber).**— In support of this view have been cited the contracted condition of the empty stomach; the periodic contraction of the empty stomach described by Boldireff in the dog; the cessation of hunger by the introduction of indigestible material into the stomach, as this is known to cause temporary inhibition of the stomach tonus; the rumbling noise (borborygmi) frequently heard in the stomach in hunger. This rumbling noise, frequently loud enough to be heard by the hungry person himself without auscultation or special attention, must be due to contractions in some region of the digestive tract.⁴

But we owe the actual demonstration of the synchrony of the hunger sensations with the strong contractions of the empty stomach

⁴ My colleague, Dr. A. B. LUCKHARDT, has called my attention to the fact that in "nervous" persons borborygmi may be very pronounced without being associated with hunger.

to Cannon and Washburn. These observers recorded the subjective sensation of hunger simultaneously with the intragastric pressure, and found that the stomach contractions and the hunger sensations run parallel. The fact that the beginning of the stomach contractions is in evidence before the hunger sensation is felt and that the sensation lasts longer than the active phase of the contraction is adduced in support of the view that contractions in some way stimulate afferent nerves in the stomach, and these impulses give rise to the hunger pangs. Cannon and Washburn also demonstrated the synchrony of the hunger sensations with similar contractions in the lower end of the esophagus.

The beautiful demonstration of Cannon and Washburn leaves undecided the nature of the stimuli causing the "hunger contractions" and the peculiar periodicity of these contractions. There can be no further question of the parallel between the stomach contractions and the hunger sensation, but the evidence for the view that the former are the cause of the latter seems to me still incomplete. I do not appreciate the force of Cannon's argument that no other condition than the contractions as the cause can account for the periodicity or intermittency of the hunger sensations. Assuming that the stomach contractions constitute the primary stimuli in the genesis of hunger, does that really solve the problem of periodicity? It would seem that the problem is only shifted a little; for these stomach contractions must depend on corresponding rhythmical activities of central or peripheral nervous mechanisms. Such a nervous rhythm giving rise to the hunger sensations indirectly through contractions in the digestive tract is just as difficult to explain as a similar nervous rhythm giving rise to or constituting the hunger sensation directly.

2. Without having done any special work in this field, the writer has for a number of years inclined to the view that motor activities are an expression or a result of the state of hunger rather than the cause of hunger. Considered biologically, it would seem that in motile organisms the expression or result of hunger ought to be motion or locomotion — to bring the organism into new environments and thus increase the chances of obtaining food. For this purpose movements of the legs, wings, fins, or cilia would be more serviceable, of course, than movements of the digestive tract. This locomotor action as an unconscious expression of hunger is beautifully illustrated in decere-

brated mammals and birds. The reader will recall Goltz's classical experiments on decerebrated dogs. Prolonged absence of food and hence a state of hunger led to restlessness and incessant locomotion in the dog without the cerebrum, while, other things being equal, a filled stomach resulted in rest and quiet. These phenomena are very striking in decerebrated pigeons, as most physiologists know from personal observations. Six years ago the writer kept a completely decerebrated pigeon for nearly a year. When the crop was empty, the pigeon would keep in incessant motion (walking) until fed, and even coo. This is true at least for periods up to sixteen hours. A few grains of corn or wheat put on the tongue and swallowed lead at once to repose in an attitude of sleep for a short period. The larger the meal the longer the period of repose. In view of what is known of the function of the cerebrum in mammals and birds it seems clear that these motor phenomena in hunger are primary and fundamental reflexes, or an automatism independent of conscious hunger states. The cessation of restlessness and locomotion in birds immediately following the introduction of a few grains of wheat or even a few grains of sand into the crop is capable of two interpretations. The restlessness and locomotion may be a reflex phenomenon, the primary stimulus being the contraction of muscular mechanism of the œsophagus and the stomach, which contractions are inhibited by mechanical stimulation of nerves in the œsophagus and the crop. Or the locomotion is an expression of a fundamental automatism inhibited by afferent impulses from nerves distributed to the crop. There has been no decisive evidence in favor of either of these two hypotheses.

II.

The subject of the present experiments, Mr. V., was not told of the nature of the experiments till near the end of the series. This was done to avoid errors from conscious interference, both in the way of inhibition and excitation. When seated or lying down during the experiments, his position was such that he could not see the kymograph or any of the recording apparatus. The signal key or keys for recording the hunger sensation were placed in his hand, and he was instructed to press the key as soon as he felt hunger and to keep on pressing it till the hunger was no longer felt. There was no difficulty

in keeping his attention fixed on this for shorter periods of one or two hours and under conditions of hunger of moderate intensity. But when the observations were continued without interruption for five to six hours and therefore during several periods of intense hunger, V. would usually become restless, apparently somewhat tired, and therefore unable to give undivided attention to the introspection. It may be stated that the man had no previous training in introspection or subjective analysis.

Most of the observations were made in a period of from four to ten hours after meals, and only a few as long as twenty-four hours after a meal, for the reason that the hunger pains in most instances became gradually more severe to the point of discomfort, and the man became restless and tired.

As a check on the intragastric respiratory pressure, records of the respiratory movements (chest pneumograph) were always taken simultaneously with that of the stomach movements.

III.

1. *Confirmation of the observations of Cannon and Washburn.* — The general results are in complete accord with those of Cannon and Washburn. When the empty stomach showed strong contraction, V. invariably signalled that he felt hunger, and on being questioned he invariably replied that he felt the hunger in his stomach. There is, on the whole, a fairly close correspondence between the duration of stomach contractions and duration of the subjective sensations of hunger. On days when the stomach did not exhibit strong contractions, V. stated that he did not feel hungry, even though in two cases the observations were continued to within twenty-four hours after the previous meal. These "hunger contractions" of the empty stomach are primarily those of the strong periodic rhythm described and illustrated in the first communication, so that further discussion at this time is superfluous.

2. *The relation between the strength of the stomach contractions and the intensity of the hunger sensations.* — Data on the above point were obtained in the following manner. Three signal magnets were arranged to record on the drum perpendicular to the recording point of the bromoform manometer, and the three corresponding keys

placed in V.'s hand. He was then instructed to press key No. 1 when he felt, without question, even the faintest hunger; No. 2 when he felt hunger of moderate strength; and No. 3 when he felt the strongest hunger. In view of the fact that V. had no training in subjective analysis, the results are remarkably uniform and accurate.

In general V. would press key No. 1 (weak hunger) at the beginning of a contraction period when the individual contractions were relatively feeble. Then, as the contraction increased in strength, there came a period of vacillation between key No. 1 and key No. 2 (moderate hunger). As the contractions grew still stronger, key No. 2 would be used for a while without any change. Then followed a period of alternation between key No. 2 and key No. 3, and in the final stage of maximum activity of the contraction period the signal was made with key No. 3 exclusively. In other words, *there is a fairly close correspondence between the strength of the stomach contractions and the degree of hunger sensations experienced simultaneously.*

The above account applies particularly to the first hunger period appearing after a meal and for the milder hunger periods in general. On more prolonged fast, that is, after having experienced several hunger periods, V. usually did not signal with key No. 1 at all, and sometimes not even with key No. 2, but would start in with key No. 3 (strongest hunger) at the very beginning of a period, despite the fact that *the strength of the stomach contractions was not greater (or might be even less) than that designated as very mild or moderate hunger a few hours earlier.* This seems to indicate either an increased excitability of the afferent nerves in the stomach, or an increased excitability of some parts of the brain.

The close parallel between the degree of the stomach contractions and the intensity of the hunger sensations is further shown by the fact that the beginning of a strong contraction was frequently signalled by key No. 2 (moderate hunger), and then a shift made to key No. 3 (strong hunger) nearer the apex of the contraction. This is illustrated in Fig. 1. *Evidently V. was able to distinguish a gradually increasing intensity of the hunger sensation during and parallel with the individual stomach contraction.* But this distinction was never made in very strong hunger and corresponding contractions. Very rarely was the beginning of a strong contraction signalled with key No. 1 (weak hunger). Whenever this happened, V. would shift to

key No. 2, and finally to key No. 3 *pari passu* with the increase in the amplitude of the contraction.

The weaker contractions of the stomach that appear between the stronger contractions during a period of moderate hunger or at the beginning of a period of strong hunger are nearly always correctly signalled with key No. 1 or key No. 2, usually the latter. This discrepancy must be noted, however, that these weaker contractions always recognized and usually signalled with key No. 2, may not show a greater degree of shortening of the muscle than the contractions present during the pauses between the strong hunger periods, and these latter contractions are usually not recognized even as mild hunger. This is a further indication of a change either in the brain or in the excitability of the nerves of the stomach during the periods of strong hunger.

3. Fusion of the hunger sensations into hunger tetanus parallel with strong and rapid contractions or tetanus of the stomach contractions. — The essential features and conditions of the incomplete tetanus of the stomach contraction at the end of the period of very vigorous contractions were described in the first communication, to which the reader is referred. These tetanus periods of the stomach are invariably accompanied by a similar fusion or tetanus of the hunger sensation. The fusion of the hunger sensation appears to be more complete than the fusion of the stomach contractions. When the rate of the strong stomach contractions approaches two per minute, the fusion of the hunger sensations is practically complete. V. is at least unable to distinguish any rhythmical variations in the hunger intensity. These phases of the stomach contractions are always signalled with key No. 3 (strongest hunger). A typical record illustrating this tetanus

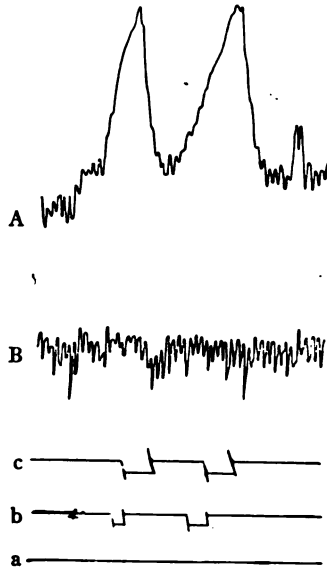


FIGURE 1. — Two thirds the original size. A, tracing of stomach contractions; B, respiratory movements; a, signal for weak hunger; b, signal for moderately strong hunger; c, signal for strong hunger. Showing the increase in the intensity of the hunger sensation during single contractions.

nus of hunger parallel with the gastric tetanus (usually incomplete) is reproduced in Fig. 2. The greater fusion of the hunger sensations than is shown by the synchronous stomach contractions is probably due to the fact that the strong individual sensations lag or persist longer than the corresponding stomach contractions.

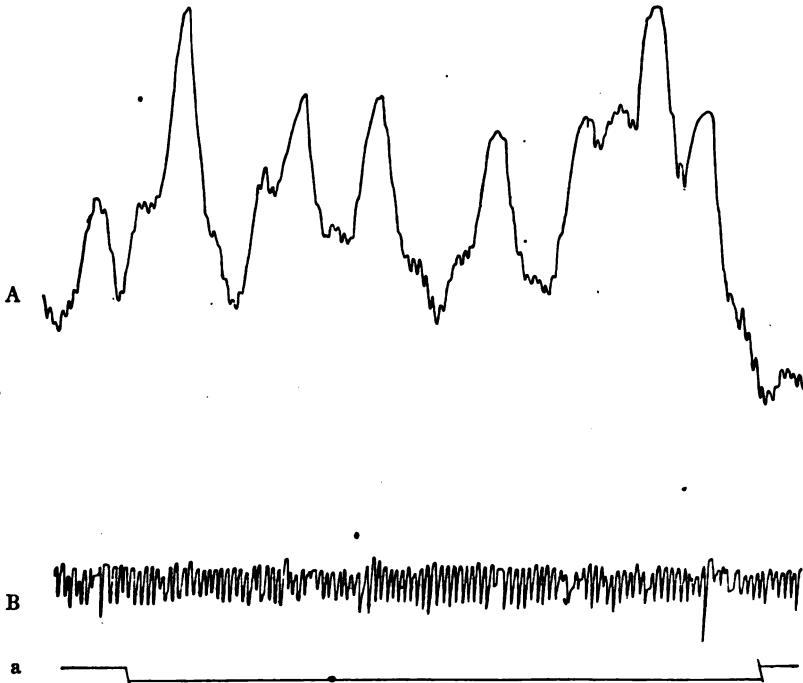


FIGURE 2. — Four fifths the original size. *A*, stomach contractions; *B*, respiratory movements; *a*, hunger signal. Showing complete fusion of the hunger sensations (hunger tetanus) during incomplete tetanus of the stomach contractions.

The abrupt cessation of the gastric tetanus at the end of a strong contraction period is accompanied by an equally abrupt and complete cessation of the hunger sensations (Fig. 3).

4. *The recognition of individual contractions of the "twenty-seconds rhythm" as "hunger contractions."*—The special character of the "twenty-seconds rhythm" of the empty human stomach is sufficiently described in my first communication. It will suffice to restate that it is not yet decided whether this rhythm, which is continuous, is due to the antrum or is also characteristic of the body of the stomach. This

seems of particular importance in connection with the fact that even these contractions may induce hunger states.

The individual contractions signalled as mild or moderate hunger are usually stronger than those not definitely recognized in consciousness (Fig. 4). But occasionally there may be no marked difference in the

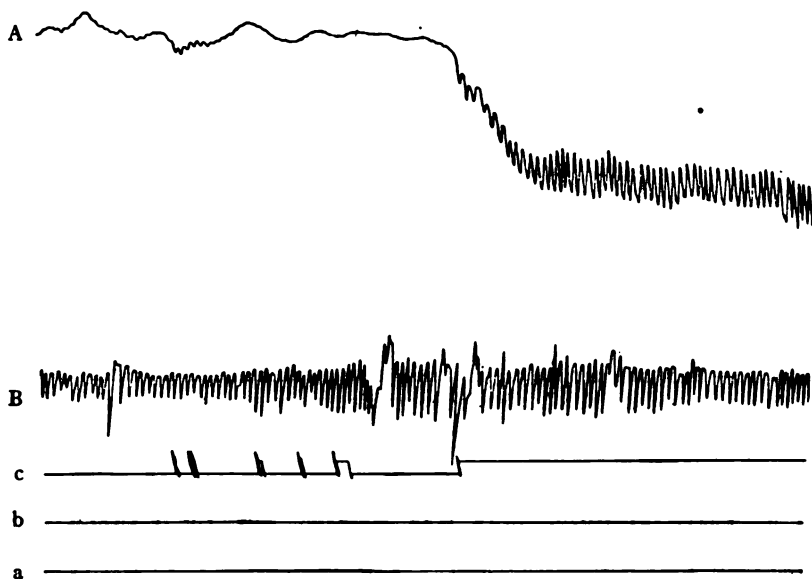


FIGURE 3. — Four fifths the original size. *A*, stomach contractions. *B*, respiratory movements; *a*, *b*, *c*, signals for weak, moderate, and strong hunger respectively. Record of the end of a period of strong hunger and hunger tetanus, showing abrupt cessation of the hunger sensation parallel with the abrupt cessation of the stomach tetanus.

amplitude of the contractions. Each consecutive contraction of the "twenty-seconds rhythm" is never signalled as a hunger contraction unless the contractions are very strong, in which case they can hardly be distinguished from the moderate contraction of the periodic or "thirty-seconds rhythm."

Assuming that the "twenty-seconds rhythm" is an antrum rhythm, and that the stomach contractions cause the hunger sensations, it follows that strong contractions of the pyloric region should cause hunger. Now, such strong contractions of this region of the stomach occur during vomiting, yet vomiting is, to my knowledge, never accompanied by hunger sensations. Of course, it is possible that the change in the physiological condition of the gastric nervous mech-

anism usually present in vomiting may account for the absence of hunger.

The recognition of only an occasional contraction of the "twenty-seconds rhythm" as a hunger contraction when all the contractions are of nearly uniform intensity is probably due to variations in attention.

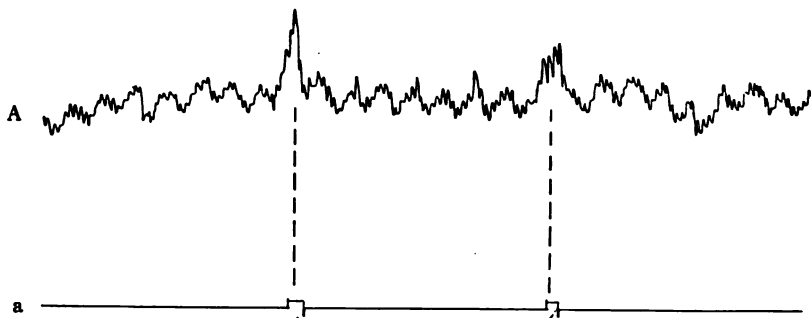


FIGURE 4. — Four sevenths the original size. *A*, stomach contractions (twenty-seconds rhythm); *a*, hunger signal. Showing recognition of the stronger contractions of the twenty-seconds rhythm as hunger pangs.

5. **The significance of the time relations between the stomach contractions and the hunger sensations.** — It was pointed out by Cannon and Washburn that the time relations between the stomach contractions and the hunger sensations might serve to determine the nature of their causal relationship. It is doubtful, however, whether the data secured by the methods so far employed are of much significance as regards this point. Unless the balloon in the stomach completely fills the stomach cavity and the pressure in the balloon is very slight, it is clear that the manometer does not register the very beginnings of the contractions. And on the subjective side we have the fluctuation of attention as a source of error. Nevertheless, a number of days were devoted to this phase despite these obvious defects in our method.

The recognition of a stomach contraction as a hunger pang depends not only on the strength of the contraction, but also on the rapidity of development of the contraction phase; that is, two contractions may indicate equal degrees of shortening of the stomach musculature, but if the contraction phase of one covers a minute or more while that of the other half a minute or less, the latter contraction only is accompanied by a definite hunger sensation. The stomach may thus exhibit slow

tonus undulations of considerable magnitude without any attendant hunger state. The reader will recall that the above relation of the rate of the contraction to the hunger sensation is in accord with one of the general "Laws of Stimulation." The fact would seem to strengthen the view that the sensation is the result of the contraction.

In no instance out of the numerous tests made did V. indicate feeling hungry *before* the beginning of the stomach contractions. But when the balloon and the manometer were adjusted as delicately as possible, the hunger signal and stomach contractions appeared nearly simultaneously. But inasmuch as the manometer probably does not register the very beginning of the contractions, it is evident that some seconds of the contraction phase always precede the hunger feeling.

When the stomach contraction is of moderate strength and hunger sensation of correspondingly moderate intensity, the hunger sensation usually ceases at the height of the contraction, but when the contractions are very strong the hunger sensation persists also during the relaxation phase. In other words, the sensation lags both at the beginning and at the end of the contraction.

6. The relation of "hunger" to "appetite." — During the intervals of the strong contraction periods, when the keys for the hunger signals are left undisturbed despite the presence of feeble rhythms in the stomach, V. was frequently asked if he would like to eat or if he felt hungry. His answer was invariably that he would like to eat or felt a desire for food, and not infrequently he would say, "I feel hungry, but I don't feel the contractions." When pressed for an explanation of this statement, he always insisted that he "felt this hunger in the stomach." The explanation of this situation is not very clear to me. The results reported in the previous pages show conclusively that the strong stomach contractions are recognized as hunger pangs, and that the rhythms of the stomach movements and the hunger sensations run parallel. Now, is the weaker but more or less persistent "hunger," referred to the epigastrium, but not definitely associated with the stomach contractions, even though the stomach shows some activity, due to confusion of *appetite* with *hunger*? Or are the feeble stomach contractions correlated with or capable of giving rise to a feeble but rather persistent hunger sensation? V. is not able to make any definite distinction between hunger (Bohemian, *hlad*) and appetite (Bohemian, *chut*). This does not appear to be due to unfamiliarity with

the language, because through an interpreter he is equally unable to make a clear distinction in his native language. This is not surprising in view of the fact that the terms "hunger" and "appetite" are used interchangeably by most people, including physiologists. Howell⁵ states that "hunger in its mild form is designated as 'appetite,'" and on this basis appetite in its strong form would be designated as hunger. Cannon and Washburn support the opposite view, that hunger and appetite are fundamentally different sensations, appetite having an agreeable character depending on previous sensations of taste and smell of food, that is, on certain memories, while hunger is essentially a painful sensation referred to the epigastrium and not dependent on previous experience with food.

According to this view, hunger is the more fundamental and primitive sensation, while appetite requires a nervous organization capable of associated memory. Is this conception of appetite adequate? Must we not look for the primary basis of appetite in a desire for food as the expression of an inherited mechanism, primarily independent of, but subsequently modified by, the individual experience? In other words, have we not in appetite for food conditions as primitive and essentially fixed by inheritance as in the case of the sexual desires or "instincts"? Pure hunger, not accompanied by "appetite," can be experienced, if during hunger the attention is fixed on the hunger pangs themselves, or if it is occupied with other processes that will completely exclude the ideas of foods and eating and their associations. When this is done, hunger in its various stages becomes different degrees of pain. The cry of the new-born child for food is, in all probability, due to this pure hunger pain (plus the inherited "desire for food") and the quieting effect of feeding due to its abolition. Pain as such, however, has no special correlation with the feeding reflexes.

The elements in appetite due to the individual's experience with foods can be experienced in the absence of hunger only, as in the contemplation of one's favorite dish shortly after a full and satisfying meal. The sensations aroused by this contemplation are agreeable and may even lead to salivation, but these sensations are not (or at least not necessarily) associated with a desire to eat. The inheritance factor in appetite, the desire to eat, is in some way caused by the hunger

⁵ HOWELL: Textbook of physiology, 1911, p. 285.

pains. This desire minus the individual's memories of previous experience with foods appears to be in evidence in extreme hunger states when unpalatable or disgusting foods, straw, sticks, etc., are chewed and swallowed.

In the adult, in normal health, the hunger pains, the desire for foods, and pleasant memories of the taste and smell of foods are ordinarily present simultaneously; they mutually reinforce one another, and lead to a common goal, the taking of food. These facts probably account for the common view that hunger and appetite designate different degrees of the same sensation.

The mutual "reinforcing" or "facilitation" action of the two sensations is seen in the universal experience of increased appetite, or the initiation (more correctly the recognition) of hunger by seeing, smelling, or tasting palatable food. It can be shown on V. that this sudden recognition of or increase in the intensity of the appetite sensation has no counterpart in a sudden initiation or increase in the hunger contractions of the stomach.⁶ The phenomena are evidently concerned with central processes, the association processes initiated by the olfactory and visual impulses affecting the paths and centres concerned in conscious hunger in such a way that subconscious hunger states enter consciousness.

If the above analysis of the elements of appetite and hunger approach the actual conditions, there appears to be no contradiction in V.'s statement of "feeling hungry," but not feeling the contractions, or hunger pangs. If during relative quiescence of the stomach the attention is not fixed on food or eating, one does not "feel hungry," because the feeble hunger processes remain subconscious. But when the attention is fixed on foods and eating, the cerebral processes involved in their memories render the weak hunger state conscious, one experiences a peculiar uncomfortable feeling of tension (not distinct pain) in the epigastrium, and the ear is cocked for the dinner bell. In strong hunger the sequence of events is reversed, the strong stomach contractions giving rise to the hunger pains and the desire for food, which in turn starts the memory processes of taste and smell of foods.

7. *The stomach contractions give rise to the hunger sensations.*—The consideration of the cause of the hunger contractions will be taken

⁶ Later work on dogs seems to show that the seeing and smelling of foods lead to strong contractions of the oesophagus.

up in a later paper, but the simpler question of the action of the contractions may be briefly dealt with now. Assuming, for the present, that the stomach contractions give rise to the hunger sensations through the action of afferent nerves from the stomach, in what way does the contraction act as the stimulus to these nerves? Does the hunger sensation arise (1) from the stimulation of nerves in the mucosa; (2) from the stimulation of nerves in the muscular coats and in the connective tissue; (3) or is it due to an inter-central discharge from the Auerbach's plexus to the brain, associated with the motor discharge from the same plexus to the stomach musculature?

As regards the first possibility, the following experiments have been made, with negative results. It would seem that the only way in which contraction of the stomach musculature could stimulate nerve endings in the mucosa is by *mechanical pressure*. This I have tried to imitate in the following way. During the period of relative quiescence of the stomach between two periods of strong hunger, when the afferent nerves concerned are in such condition that their stimulation will give rise to hunger, mechanical pressure on the mucosa by distention of the balloon or rubbing the mucosa by the closed end of a test tube never causes sensations of hunger unless these procedures lead to contraction. V. always stated that he felt these pressures, but the sensations were not like hunger. The objection might be raised against these experiments that the pressure is not sufficiently strong, and, in the case of the test tube, does not touch a sufficiently large area of the mucosa. I admit that a more intense mechanical stimulation of the mucosa could be produced by Pawlow's method of blowing sand into the stomach by bellows. But I have not felt justified in using similar procedures on V. The methods used do not, of course, produce the strongest possible mechanical stimulation of the mucosa, but these stimulations were sufficient to affect consciousness. They were perceived, but not as hunger sensations. It seems therefore highly probable that the afferent nerves in the mucosa are not primarily concerned in the genesis of the hunger sense. *They are, however, concerned in the inhibition of hunger.*

The hunger sensation seems to be produced by the contractions only. When the empty stomach is normal, strong contractions, however caused, cause hunger. Thus, if the balloon in the stomach is rather suddenly distended, this may produce one, two, or three

strong contractions of the previously quiescent stomach, and these are recognized as hunger contractions identical with those of the "spontaneous" hunger periods. It seems to me that this experiment constitutes a *demonstration of the peripheral genesis of hunger*, as the subjective state clearly is induced by the peripheral change. A tracing illustrating this phenomenon is reproduced in Fig. 5.

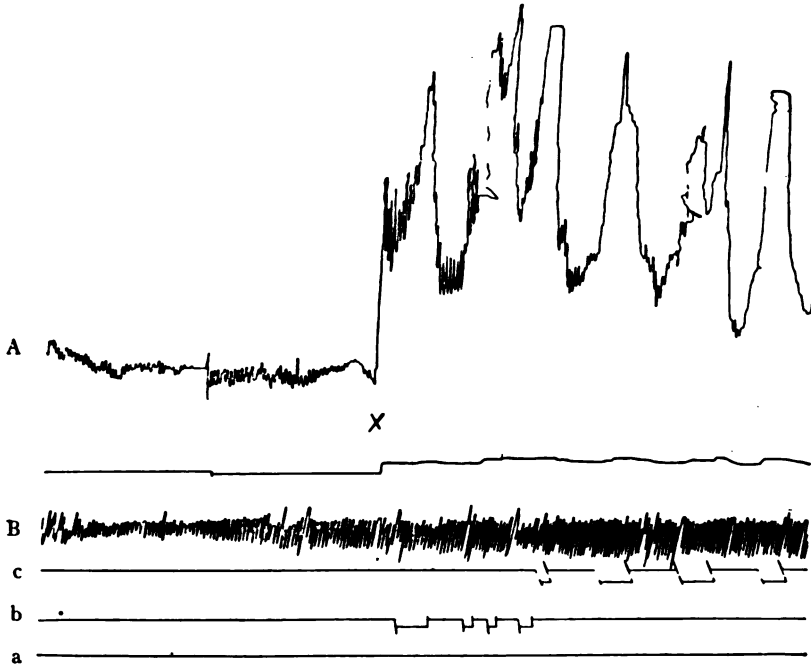


FIGURE 5. — Two thirds the original size. *A*, stomach contractions; *B*, respiratory movements; *a, b, c*, signals for weak, moderate, and strong hunger respectively. The pressure in the balloon is slight. There is no evidence of strong stomach contractions, and *V.* feels no hunger. At *x* the pressure in the balloon is suddenly increased. This distention of the balloon initiates a few strong stomach contractions, which in turn cause the hunger states. A demonstration of the gastric genesis of hunger.

But how do the contractions stimulate the afferent nerves in the muscle layers? None of my observations on *V.* throw any light on this question. Contraction in skeletal muscle stimulates afferent nerve fibres in the muscle. But it seems to me that the pain experienced from contractures or "cramps" in skeletal muscles and in the intestines is different from the hunger pangs, even though pain is in-

herent in hunger. The difference may be only an apparent one, due to the fact that the latter pains arouse the memories of previous agreeable experiences with food. Because of the folding of the mucosa and the sub-mucosa into rugæ and the changes in the arrangement of the cells in the muscle layers in the stomach during contractions,⁷ there must be a great variation in tension on the nerve fibres in the contracted and in the relaxed condition of the stomach wall. This variation in tension, rather than actual pressure, may constitute the stimulus, in so far as the stimulus is a mechanical one. It would therefore seem that hunger contains elements of kinæsthetic sensation as well as pain, the latter predominating in strong hunger.

8. I have been strongly impressed by V.'s ability to recognize feeble stomach contractions as hunger states. It is probable that very strong stomach contractions can be recognized as separate hunger pangs by most men. The writer has at least no difficulty in doing this. But the delicate analysis disclosed in these experiments on V. is beyond the writer's ability in introspection. On many days I denied myself food for periods similar to those imposed on V. so as to bring the stomach of subject and observer in conditions as nearly alike as possible. But even with the stomach rhythm and the hunger rhythm of V. being recorded before my eyes and convinced that my own stomach was engaged in a similar rhythm, as hunger was present, the most that I could determine was the fact that the moderate hunger sensations were more or less discontinuous.

Two explanations of V.'s unusual ability in recognizing the stomach activities have occurred to the writer. (1) Since early boyhood the stomach has been to V the object of special care and attention. In consequence of this special attention to the stomach the afferent nervous impulses from the stomach may have attained a clearer definition in consciousness analogous to the remarkable development of analysis in the tactile or pressure senses in the absence of vision. If this hypothesis is correct, afferent impulses from the stomach other than those concerned in hunger ought to show a similar marked influence on consciousness in V. (2) At the point of the gastrostomy V.'s stomach adheres to the parietal peritoneum. There may be adhesions of greater extent in consequence of the operation. The hunger

⁷ MÜLLER, cited from CANNON: *The mechanical factors in digestion*, 1911, p. 60.

sensation of V. may therefore include a greater degree of pain than is the case in normal men, as the contracting stomach may pull on the parietal peritoneum, which, according to many observers, is very sensitive to painful stimuli. The weaker stomach contractions may thus be recognized as hunger because more painful than under normal conditions. I have questioned V., but he is unable to recollect any

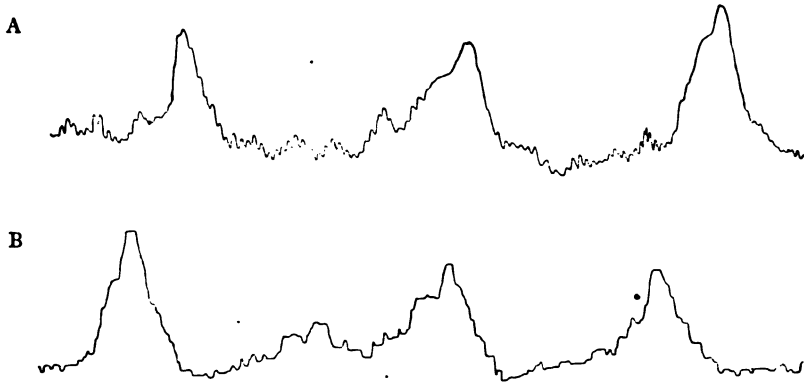


FIGURE 6. — Two fifths the original size. *A*, tracing of the hunger contractions of the empty stomach in man (Mr. V.); *B*, tracings of the contractions of the empty stomach of a dog with a gastric fistula. Showing practical identity of the rhythms of the empty stomach in man and dog.

difference in his hunger sensation before his accident and after the completion of the gastrostomy. Confirmatory evidence (or the opposite) ought to be obtainable without much difficulty, as cases of gastrostomy are fairly common. Clinicians having such cases in hand would do a service to physiology if they could determine whether gastrostomy invariably augments the hunger sensations or makes the hunger pangs more painful.

Two other possibilities have been suggested. There may be an increased excitability of the afferent nerves of the stomach as a result of the constant presence of the rubber tube. But there seems to be no evidence of this in the appearance or in the sensibility of the gastric mucosa. My colleague, Professor Lingle, suggested that cutaneous sensory nerve fibres may have grown into the stomach wall in consequence of the union of the stomach and the body walls. In such case there may be elements of epicritic pain and touch in V.'s hunger pangs. This seems improbable in view of what is known of the spe-

cific character of the regeneration of nerve fibres after lesion, and I have not been able to obtain any "tactile" sensations from the gastric mucosa in proximity to the gastrostomy.

9. The similarity of the hunger contractions of the empty stomach of man to the strong contractions of the empty stomach in the dog. — The work on dogs with a fistula in the fundus for the introduction of the balloon as originally employed by Boldireff has been carried far enough to establish the practical identity in character of the contractions of the empty stomach in man and dog. The rapid or continuous rhythm shown by the stomach of V. is also shown by the dog's stomach. The rate of the rhythm is different, however. But it is the strong stomach contractions that are of interest in this connection. When the size of the balloon is adjusted approximately to the difference in capacity of the human and canine stomachs, the records obtained from man and dog can scarcely be told apart (Fig. 6). The man tells us that a sensation of strong hunger is felt synchronously with the contraction. We have a right to conclude that the dog experiences the same sensation simultaneously with the corresponding contraction. *We have, then, in the strong contractions of the empty stomach an objective criterion for the presence or absence of hunger in experimental animals.*

It may be noted in this connection that dogs will show a lively interest in and great desire for food during periods when the strong contractions of the empty stomach are absent. And conversely, if the dog suspects that he will be allowed to see and smell the food only, he may show no interest in the food, despite the presence of strong stomach contractions with the attendant strong hunger sensations. Interest in and desire for food are therefore no criterion for hunger in experimental animals.

THE EFFECTS OF AORTIC COMPRESSION ON THE CIRCULATION.

BY TORALD SOLLMANN AND J. D. PILCHER.

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THE gross effects of compression of the aorta on the blood pressure are well known. They consist (Fig. 1) in a considerable and well-sustained rise, followed, when the pressure is released, by a brief fall and complete recovery. The present investigation was undertaken primarily to study the behavior of the vasomotor centre under these conditions, and to ascertain whether this gives any evidence of physiological stimulation of the depressor mechanism, which is commonly supposed to be excited by increase of intracardiac or intra-aortic pressure. The result being negative, the investigation was extended to the influence of various modifying conditions, and to the behavior of the heart.

I. EFFECTS OF AORTIC COMPRESSION ON THE VASOMOTOR CENTRE.

The response of the vasomotor centre was studied by the perfusion method previously described;¹ namely, by the artificial perfusion of an organ in a living animal, with the nervous connections intact, but completely separated from the circulation of the animal. Otherwise the technic varied somewhat in the different experiments. Cats and dogs were used. The cats were anesthetized by our atropin-morphin-ethyl carbamate mixture, the dogs with morphin and ether. Some of the dogs also received atropin. Most of the animals were immobilized by curare, artificial respiration being supplied by bellows or by oxygen insufflation. The compression of the aorta was generally made by the finger, introduced through a small opening of the thorax,

¹ SOLLMANN and PILCHER: this Journal, 1910, xxvi, pp. 233-238.

which was thus kept closed. The vagi were sometimes intact, sometimes divided. Several compressions, generally of about one minute

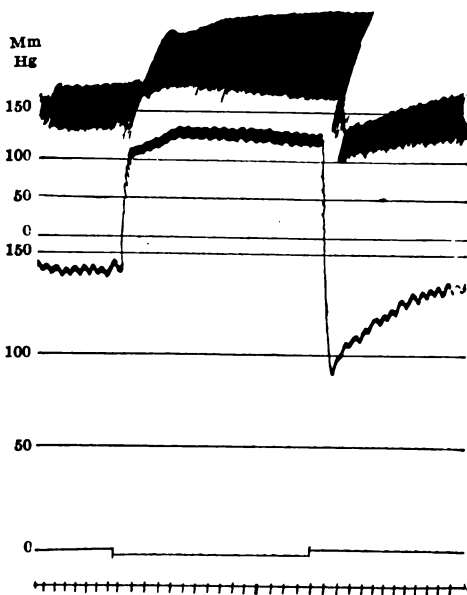


FIGURE 1. — One half the original size. Compression and release of the aorta on carotid blood pressure. Upper tracing, membrane manometer; second tracing, damped mercury manometer. Second line from bottom, abscissa for blood pressure, and signal during which aorta was clamped, just above diaphragm, for two and one-half minutes. Bottom line, flow from perfused spleen, showing constriction (slowing) (Dog C41-2-9). The lower ordinates show the mercury manometer pressures from 0 to 150 mm. Hg; the upper ordinates show the pressures for the membrane manometer.

duration, were made on most of the animals, also some longer compressions, which will be described separately.

Vasomotor response to brief compression. — Of six atropinized cats, in four (C74, 77, 99, 101) the spleen responded by vasoconstriction (slowing of the perfusion flow); one (C76) showed no effects; one (C100) showed vasodilation. Of eight dogs, five (spleens C41, 44, 47, 57 and kidney 94) responded with constriction, and three (spleens C42, 43, 141) with dilation. The usual result is therefore vasoconstriction (Fig. 1). The degree of this constriction is quite moderate for cats, the slowing of the perfusion flow averaging about $8\frac{1}{2}$ per cent. It is more pronounced in dogs, the average slowing of flow being 35 per cent. The excitation is less powerful than that of asphyxia, for a

number of animals which failed to react to aortic compression still showed slowing during asphyxia.

The vagi do not influence the vasomotor response materially, for the response was the same whether the vagi were intact or divided.

Of five cats with vagi intact, four showed constriction and one was negative.

Of two cats with vagi divided, one showed constriction, the other was negative.

Of six dogs with vagi intact, four showed constriction, two dilation.

Of two dogs with vagi divided, one showed constriction, one dilation.

The vasomotor response is also essentially independent of the original level of blood pressure, above 50 mm. With lower blood pressures the response is by dilation.

Grouping our experiments according to the level of blood pressure just previous to compressing the aorta, we find with:

Blood pressure below 45 mm., dilation in one cat and in one dog.

Blood pressure between 50 and 80 mm., constriction in one cat.

Blood pressure between 85 and 140 mm., constriction in four dogs, dilation in one dog.

Blood pressure above 145 mm., constriction in three cats and one dog, negative in one cat.

Vasodilation from aortic compression (influence of asphyxia). — Of all our experiments, vasodilation occurred only in three dogs and once in a cat. The examination of the tracings in all of these dilating animals shows that the flow was markedly slowed, and therefore the vasomotor centre was markedly stimulated, *before* the aorta was compressed. This preliminary constriction was due to inefficient respiration in two of the dogs (C42 and 43), and to excessively low blood pressure and consequent anemia of the centre in the third dog (C141, blood pressure, 22 mm.), and in the cat (C100, blood pressure, 40 mm.). The vagi were not concerned in the phenomenon. They were intact in Dogs 42 and 43, divided in Dog 141. In the cat the same result was obtained before and after their division.

The dilation in all of these cases would seem to be due to the relief of the asphyxial or anemic stimulation of the vasomotor centre by the increased blood supply, when the aorta is compressed. This explanation is confirmed by the observation that tracings of all the other experiments, in which the aortic compression produced vasoconstriction, fail to reveal any evidence of asphyxial or other pre-existing vasomotor stimulation.

Release from brief aortic compression. — The changes in the vasomotor centre tend to return quite promptly to the normal when the compression of the aorta is released. If the compression slowed the flow,

this is again quickened to about the original rate — sometimes slightly above or below. If the flow was quickened by compression, it is slowed to about the normal on release. When compression produced no changes, release often caused a little slowing — perhaps as a delayed response to the compression.

The vasomotor centre is therefore not concerned in the characteristic brief fall of blood pressure below the original level, which normally occurs immediately after the aorta is released.

Repeated compression of the aorta (up to six or seven compressions of about one minute duration, at short intervals) does not markedly change the vasomotor response.

Thus, in Dog 44, the first compression raised the blood pressure from 90 to 170 mm. and slowed the flow from 33 to 30 units. The sixth compression raised the pressure from 70 to 158 mm. and slowed the flow from 20 to 18 units.

In Dog 47 the second compression raised the pressure from 135 to 160 and slowed the flow from 63 to 20. The sixth compression produced a rise from 135 to 170 and a slowing from 80 to 40.

Continuous compressions of the aorta for longer periods (up to an hour) were tried in a number of animals; unfortunately, these did not happen to show a good vasomotor response. We may say, however, that the constriction does not grow any weaker within six minutes; it may remain unchanged (C47), or grow slightly stronger (C44 and C57).

Explanation of the vasomotor response. — *A priori*, several factors might enter into the response of the vasomotor centre to aortic compression. The following seem to be the most important:

1. The increase of intracranial pressure, which, as is well known, produces vasoconstriction. This seems to explain the normal constrictor response which we have described.

2. The increased blood flow to the brain may relieve asphyxial constriction. This we have also described.

3. The heart and aorta would try to relieve themselves of the excessive pressure by stimulation of the depressor nerve, thus producing vasodilation. This was the function assumed for this nerve by its discoverers, Cyon and Ludwig, and their view is still currently accepted. We ourselves expected this result, since we had shown

in a previous paper² that our method responds well to depressor stimulation. Our failure to demonstrate vasodilation on aortic compression shows, therefore, that this procedure does not stimulate the depressor mechanism effectively unless, indeed, the stimulation be merely momentary, for our method is not well adapted to reveal momentary changes.

The evidence for the normal function of the depressor nerve. — This unexpected result led us to review the evidence on which the current view of the normal functions of the depressor nerve is based. We may say at once that this is surprisingly meagre and unsatisfactory.

Cyon and Ludwig apparently based their suggestion on the anatomical relation of the depressor nerve, and the assumed physiological benefit of such a safety-valve device to the heart.

Their only experimental work in this connection was really negative, for they found that section of both depressor nerves (in rabbit) did not raise the blood pressure, indicating that the depressors are not tonically active, whatever may be their normal stimulus.

This experiment has been repeated by many subsequent workers, with contradictory results: Sewall and Steiner³ generally found a rise of 1 to 3 cm. Hg, on dividing the depressors in rabbits; Bayliss⁴ obtained no rise in normal rabbits, but did obtain a rise of 1 to 6 cm. of Hg in animals which had received large injections of saline solution. Hirsch and Stadler⁵ confirm Sewall and Steiner, finding that the pressure rose, on section, by 11 to 34 mm., the rise being maintained for eight to fifteen minutes. They attribute the negative results of Cyon and Ludwig and of Bayliss to injury of the nerve by trauma and exposure. Contrary to Bayliss (and to the results of Pawlow⁶ on division of the vagi in dogs), they find that the rise is no greater after transfusion.

The data indicate that section of the depressor nerves may raise the blood pressure, and that they are therefore tonically active, at least at times.

The evidence that the depressor nerve is actually stimulated by

² SOLLMANN and PILCHER: this Journal, 1912, xxx, p. 369.

³ SEWALL and STEINER: Journal of physiology, 1885, vi, p. 162.

⁴ BAYLISS: *Ibid.*, 1893, xiv, p. 314..

⁵ HIRSCH and STADLER: Deutsches Archiv für klinische Medizin, 1904, lxxxi, p. 391.

⁶ PAWLOW: Archiv für die gesammte Physiologie, 1879, xx, p. 210.

rise of blood pressure is much less satisfactory. It has been attempted mainly by comparing the rise produced by various methods, before and after section of the depressor nerves.

Sewall and Steiner³ claimed that the rise of pressure which occurs on clamping both carotid arteries is considerably larger if the depressor nerves have been divided. This is contradicted by Bayliss,⁴ but confirmed by Hirsch and Stadler.⁵

Sewall and Steiner also claimed that when the aorta is compressed and then released the primary fall is followed by a secondary rise if the depressors have been divided. This they attribute to anemic stimulation of the vasomotor centre by the low blood pressure, which is prevented by the depressor nerve if this is intact. (It seems to us that this would be contrary to the assumed function of the depressor, namely, the relief from excessive pressure. Moreover, our numerous tracings fail to confirm the observation; we have observed the secondary rise about as often when the depressors were intact as when they were divided.)

Bayliss,⁴ on the other hand, finds that a given degree of asphyxia produces the same rise of pressure, whether the depressors are intact or divided. The high asphyxial rise of pressure should certainly stimulate the hypothetical function of the depressor, but it may be objected that the powerful vasoconstrictor stimulation may completely obliterate the weaker dilator stimulation; this is actually the case in artificial depressor stimulation, as shown by Bayliss.

Hirsch and Stadler⁵ finally attempted to evoke the depressor function by experimental cardiac lesions, but unsuccessfully; division of the depressors gave about the same rise which they had found in normal animals.

The stronger support of the current theory is furnished by Köster and Tschermak.⁷ Having shown by histological methods that the depressor endings lie in the aorta, they find that compression of the abdominal aorta or artificial pressure within the aorta gives rise to action currents in the depressor nerve. Results could generally be obtained also by electrical stimulation of the aorta, but not by longitudinal traction.

These observations (which have apparently not been repeated by others) seem flatly opposed to the results of our method. It is not impossible, however, to reconcile the two; for it is conceivable that compression of the aorta stimulates the depressor nerves, as claimed

⁷ KÖSTER and TSCHERMAK: *Archiv für die gesammte Physiologie*, 1902, xciii, p. 24.

by Köster and Tschermak, but that this stimulation is not capable of making itself felt against the more powerful vasoconstrictor stimulation of cerebral compression; just as depressor stimulation fails in intense asphyxia. The net result would be the vasoconstriction shown by our method.

The fact that the depressor mechanism fails in the presence of high blood pressure robs it of some of its assumed importance. It does not mean, however, that it is entirely useless. It may be more effective in the relatively much smaller changes of aortic pressure which are encountered under the ordinary conditions of life. Hirsch and Stadler suggest that the real function of the depressors is to regulate the blood pressure in the different phases of the cardiac cycle. As to all this, however, we have no data.

II. CONDITIONS MODIFYING THE BLOOD PRESSURE RESPONSE TO AORTIC COMPRESSION; THE RISE OF BLOOD PRESSURE ON COM- PRESSING THE AORTA AT DIFFERENT LEVELS OF BLOOD PRESSURE.

In a previous paper ⁸ we have emphasized that cardio-vascular reactions in general are influenced quantitatively by the level of blood pressure at which they are produced. This has led us to tabulate our data on compression of the thoracic aorta, as shown in Table I. In this table the results are grouped at intervals of 20 mm. of blood pressure. When several compressions were made on an animal, all those within a range of 10 mm. were averaged and counted as a single experiment. From these results the curves of Figs. 2 and 3 were constructed. In these curves the data of all the atropinized cats have been combined, whether the vagi are intact or divided, since the separate curves for these are practically identical (as may be seen from the inspection of the table). Similarly, the data for dogs with vagi divided have been combined with those of atropinized dogs in which the vagi were intact.

Restricting ourselves for the present to the animals whose vagi have been eliminated by section or atropin, Fig. 2 shows that the absolute rise on compression of the aorta is practically constant be-

⁸ SOLLMANN and PILCHER: *Journal of pharmacology and experimental therapeutics*, 1911, iii, p. 48.

tween the levels of 40 and 120 mm. In this entire range the average rise for dogs varies from 68 to 78 mm., in cats from 41 to 56 mm. (In cats there is a rather constant tendency for the rise to increase with

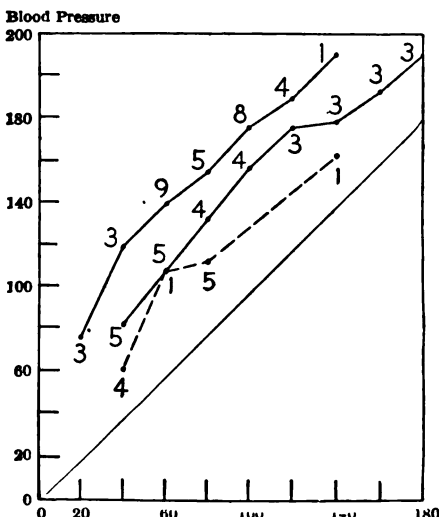


FIGURE 2. — Absolute rise of blood pressure on compressing the aorta at different levels of blood pressure. The diagonal represents the ascending level of blood pressure at which compression was made; the curves show the average level to which the blood pressure rose on compression. The vertical distance between the curves and the diagonal shows the absolute rise of blood pressure. The numerals on each curve indicate the number of experiments from which the average rise at this level was deduced. Upper curve, dogs with vagi paralyzed by section or atropin. Middle curve, cats with vagi paralyzed by atropin, with or without section of the nerves. Lowest curve, dogs with active vagi.

the blood pressure, but this is not noticeable in the dog.) Below 40 mm. and above 120 mm., the absolute rise is not as great. The decreased response may presumably be attributed to relative cardiac insufficiency, from the low blood supply in the one case, and from the excessively high pressure in the other.

Leaving these extremes out of consideration, it seems that the compression of the aorta produces quantitatively the same absolute rise at all ordinary levels of blood pressure; the percentile rise (Fig. 3), therefore, varies inversely to the level of blood pressure.

The influence of the blood pressure is therefore the same for aortic compression as for sciatic stimulation; indeed, a comparison of the aortic curves with those compiled by Porter⁹ for sciatic stimulations show that the two phenomena are strictly parallel. From this in-

crease of percentile response to sciatic stimulation, as the level of blood pressure falls, Porter argues an increased response of the vasomotor centre. We see now that the same phenomenon occurs in aortic compression, in which the vasomotor centre cannot play any essential part in the rise. This supports our previous conten-

⁹ PORTER: this Journal, 1907, xx, p. 404.

TABLE I.
AVERAGES OF BLOOD PRESSURE RISE ON COMPRESSION OF THE AORTA.

Level of blood pressure before compression.	Cats, atropin, vagi intact.			Cats, atropin, vagi divided.			Dogs, atropin, vagi intact.			Dogs, vagi divided.			Dogs, vagi active.		
	Num-ber of ani-mals.	Absol-ute rise.	Per-centile rise.	Num-ber of ani-mals.	Absol-ute rise.	Per-centile rise.	Num-ber of ani-mals.	Absol-ute rise.	Per-centile rise.	Num-ber of ani-mals.	Absol-ute rise.	Per-centile rise.	Num-ber of ani-mals.	Absol-ute rise.	Per-centile rise.
11-30	1 ¹	8	8	3	55	275
31-50	1	35	88	4	43	108	3	79	198	4	19	48
51-70	1	33	55	4	52	87	2	72	120	7	81	135	1	49	82
71-90	4	51	64	5	74	93	5	32	40
91-110	4	56	56	3	79	79	5	73	73
111-130	3	56	47	4	68	57
131-150	3	33	24	2	45	32	1	70	50	1	23	16
151-170	3	31	20
171-190	3	30	16

¹ This result is not incorporated in the curves, since it occurred at the end of an experiment, when the heart had almost failed; and it cannot, therefore, be considered as typical.

tion ¹⁰ that the percentile response is not an infallible index of the intensity of vasomotor changes, and that it does not furnish proof

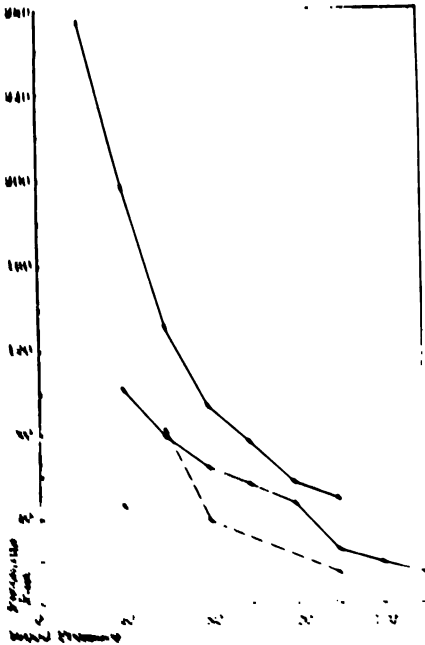


FIGURE 2. Percentile rise in blood pressure in response to aortic compression. The order of the curves is the same as in the preceding figure.

average all the data between these limits. This gives the following results for the rise in pressure.

TABLE I

1. In the active state (normal) the response to aortic compression is 100%.
2. In the active state (normal) the response to aortic compression is 100%.

TABLE II

1. In the active state (normal) the response to aortic compression is 100%.
2. In the active state (normal) the response to aortic compression is 100%.

1. In the active state (normal) the response to aortic compression is 100%.

that the vasomotor centre reacts more powerfully to sciatic stimulation as the level of blood pressure descends.

The influence of the vagi on the response to aortic compression. — Table I and Figs. 2 and 3 show that the pressure does not rise as high when the vagi are active as it does when they have been paralyzed by section or atropin. The lesser rise is doubtless due to reflex vagus slowing, although we did not make accurate observations of this point. On the other hand, there is no difference between animals in which the vagi are divided and those with vagi intact but paralyzed by atropin. Since the absolute response is the same for the range of blood pressure between 80 and 120 mm. it is possible to

These results furnish additional proof that the compression of the aorta does not effectively stimulate the depressor nerves. Were there such a stimulation, then the rise should be greater after section of the vagi depressors, since a considerable part of the vascular area is still functional when the descending aorta is clamped.¹¹

It is still conceivable that the depressors would be stimulated only when the aortic pressure surpassed the physiological limits. Choosing, somewhat arbitrarily, 150 mm. as this limit, this would be reached on aortic compression, in cats from an original level of about 100 mm., in dogs from 80 mm. We have therefore averaged the data above these limits:

CATS BETWEEN 101 AND 130 MM.

Vagi intact, but atropinized: three experiments, rise averages 56 mm.

Vagi divided: two experiments, rise averages 53 mm.

DOGS BETWEEN 81 AND 130 MM.

Vagi intact, but atropinized: six experiments, rise averages 75 mm.

Vagi divided: nine experiments, rise averages 71 mm.

Again, it is evident that the response is not increased by section of the vagi.

If, instead of dealing with general averages, we examine the individual results on atropinized cats, we find that the aortic compression rise is very often greater after the vagi are divided; but this difference is always connected with changes in the blood pressure beyond the critical level. The observations with intact vagi are naturally made at the beginning of the experiment, when the blood pressure in cats is often above the level of 140 mm., where the aortic rise averages only about 30 mm. When the experiment has proceeded to the division of vagi, the pressure has generally fallen below this level. The difference in the compression rise must be attributed to this change in blood pressure, and not the division of the vagi. Thus, in five experiments of this kind eight compressions were made with intact vagi, at an average blood pressure of 161 mm. and an average rise of 30 mm. After section of the vagi eight compressions were made, at an average pressure of 104 mm. and an average rise of 54 mm. Table I

¹¹ SOLLMANN and PILCHER: this Journal, 1912, xxx, p. 372.

shows that these figures correspond to the ordinary changes with the change of pressure and are therefore not due specifically to the division of the vagi.

This is also shown by the following experiments (atropinized cats). The rise on aortic compression was:

EXP., C76	EXP., C77
Before division, 192 ± 30 mm.	172 ± 23 mm.
Before division, 168 ± 46 mm.	160 ± 30 mm.
After division, 150 ± 53 mm.	135 ± 37 mm.

Here the change in the compression rise is quite as great before as after the division of the vagi.

Again in an experiment with low blood pressure (C101) division produced no change:

Rise before divisions, 52 ± 33 mm.
Rise before divisions, 52 ± 36 mm.
Rise after divisions, 50 ± 30 mm.

Blood pressure response to repeated compression. — In a given animal repeated compressions give remarkably uniform results, provided that the vagi are paralyzed and that the blood pressure remains within the critical limits. This holds true even when seven brief compressions are practised in close succession, as in Dog C44.

In this dog (C44, atropin, vagi intact) seven compressions, each of about one minute duration were made, with intermissions of one to five minutes. The blood pressure before the compressions ranged between 65 and 90 mm. The rise on compression ranged between 75 and 85 mm.

Similar uniformity obtained in another dog (199) with seven compressions and in four dogs each with three compressions; also in three cats with two to four compressions. In dogs with active vagi (C47 and 116) the results are naturally more variable, but in these, also, there is no indication of diminished reaction.

We have already discussed that the reaction of the vasomotor centre also remains uniform under repeated compressions.

Effect of the duration of the compression on the blood pressure. — When the aorta is compressed, the blood pressure ascends more or less sharply, reaching its maximum within half a minute to two minutes (Fig. 1). It is then maintained some twenty minutes.

Most of the compressions were terminated within five minutes, but in two dogs they were much more prolonged:

In Dog C44, atropin, seven short compressions had been made. In the eighth the aorta was left occluded. The pressure rose from 65 to 142 = 77 mm. in two minutes; it then fell very slowly, so that at the end of twenty minutes it had fallen 14 mm., namely, to 128 mm., in 25 minutes to 123 mm. Then it began to decline more rapidly, reaching 62 mm. at thirty minutes after the ligation. Injection of toxic doses of strychnin were then made into the jugular vein. Forty-five minutes after occlusion, the pressure stood 32 mm.; at seventy minutes the pressure was still 35 mm. The dog was then killed.

In Dog 47, vagi active, six short compressions had been made. The seventh was prolonged for twenty minutes. The pressure rose 130 to 167 in two minutes, maintained this level till ten minutes, then fell gradually through 14 mm. to 153 mm. at twenty minutes.

The heart is therefore capable of sustaining the relatively high pressure and other abnormal conditions of compression for at least twenty minutes. After this time it weakened progressively in the one experiment which was prolonged beyond this period. Wolfer¹² finds that the normal or hypertrophied heart may work for three hours after occlusion of the aorta, although exceptionally death may occur early.

The temporary fall of blood pressure on releasing the aorta. — When the aorta is suddenly released from compression, the blood pressure drops sharply to a variable extent, but generally some 25 or 30 mm. below the original level (Fig. 1). The pressure recovers within two to five minutes to somewhere near the normal level, often with a small preliminary rise. The temporary fall of pressure might be due to either cardiac or vasomotor disturbance. Injury to the heart by the preceding compression may be excluded, since the heart can sustain the high pressure for long periods. However, we shall show that the sudden release of the pressure disturbs the cardiac mechanism temporarily, and this would account for the fall of pressure. On the other hand, Sewall and Steiner,³ p. 167, apparently without any direct investigation, attributed the fall to peripheral paralysis of the blood vessels by the preceding anemia.

¹² P. WOLFER: *Archiv für experimentelle Pathologie und Pharmacologie*, 1912, lxxviii, p. 434.

The careful examination of our data fails to reveal any support for this assumption: *the fall of pressure is not due to vasomotor paralysis*. If vasomotor paralysis played a prominent part in the fall, then the fall should be proportional to the vasomotor tone obtaining before the compression; in other words, the higher the original blood pressure, the greater should be the compensatory fall. This does not correspond to the facts. Arranging our data according to the levels of blood pressure, we find that the absolute fall is insignificant below a certain level of blood pressure, namely, in cats about 70 mm., in dogs about 90 mm. Above these levels the fall averages practically constant, and therefore bears no relation to the degree of vasomotor tone.

Again, one would expect the degree of the hypothetical anemic vasomotor paralysis to increase with the duration of the anemia. This is not the case; it is quite as great with compressions lasting half a minute or less, as with compressions lasting five minutes or longer.

The rapid recovery of the blood pressure, even after prolonged compression (twenty minutes in Exp. C47), also speaks strongly against vasomotor paralysis, for this would scarcely pass off so promptly.

The fall does not occur if the pressure is released gradually (C199-4). This modification would not affect a vasomotor paralysis, but would prevent the sudden cardiac disturbance.

The final proof that the vasomotor mechanism is not paralyzed is the observation that the blood pressure rises promptly if the sciatic nerve is stimulated (C43-2), or if asphyxia is induced (C86). On the other hand, depressor stimulation is indeed often relatively ineffective during the fall (Sollmann and Pilcher, 2), but this need not mean that the vasomotor mechanism is paralyzed. It may be, and probably is, due to the altered distribution of the blood.

III. PULSE PRESSURE AND CARDIAC TRACINGS.

Membrane manometer records. — These were made to study the relative changes in the systolic, diastolic, and pulse pressures. Sixteen compressions were made on dogs, with inactivated vagi, attach-

ing the Harvard instrument to the carotid artery. The typical phenomena are shown in Fig. 1.

The *excursions (pulse pressure)* during the compression may be increased (seven cases), unchanged (two cases), or diminished (five cases). The average change was an increase of about 25 per cent of the normal amplitude, early in the compression, increasing to about 35 per cent after a minute. In the one experiment (C44) in which the compression was prolonged the excursion declined gradually from the normal (100 per cent) to 67 per cent in four to thirty minutes, to 33 per cent in fifty minutes.

Immediately on releasing the compression, that is, during the fall of blood pressure, the excursions are generally decreased (eight cases); in three cases they remained somewhat increased, and in three they remained normal. The average amplitude of the excursions at this period is about 90 per cent of the normal.

Some time after the release the pulse pressure returns to normal (four cases); or may be somewhat above (five cases) or below (four cases). The average amplitude is practically the normal (103 per cent).

The systolic diastolic pressures during the compression are, of course, both increased. The increase is relatively greater in the systolic pressure.

Immediately after release, both pressures fall, and the systolic fall is again the greater. *Later*, both pressures return practically to the normal.

Six compressions were also made on a dog (C47) with active vagi. The excursions were generally unchanged during the compression, and showed some increase on release. The systolic and diastolic pressure rose about equally during the compressions. On release the systolic pressure returned only to normal, whilst the diastolic pressure fell below normal.

Taking these data together, it will be seen that during the compression the heart contracts rather more forcefully, increasing the pulse pressure and the systolic pressure relatively as well as absolutely.

Immediately on release the heart is evidently working less efficiently, as evidenced by the decreased pulse pressure and the relatively greater drop of the systolic pressure. This agrees well with the view that the fall of blood pressure is due to lessened cardiac efficiency rather than to vasomotor paralysis, which would increase the pulse pressure and give a relatively greater fall in the diastolic pressure.

Cardiac tracings under aortic compression and release. — Cardiograms were taken from the ventricles by several methods. The Cushny myocardiograph was employed in four dogs and one cat, using

sometimes the left, sometimes the right ventricle; in one frog the apex of the ventricle was hooked to an ordinary lever. In two rats tracings were taken by the ampullature method through the incised thorax. A carbon-pneumatic method was used in two lings.

With the lever methods twenty-six experiments were made in eight animals. The frogs were divided or unanesthetized. Chloroform and artificial respiration were given as needed. The blood pressure changes were also recorded but showed nothing new, and need not be discussed. The typical cardiac changes are illustrated in Fig. 1.

Amplitude of excursions.—During the convulsions the changes are inconsistent, but in the whole the amplitude tends to decrease. Unchanged amplitudes were present in twelve cases, unchanged in five, increased in six. The average was about 50 per cent of the normal excursion. This slight difference and the inconsistency of the change show that it has little significance.

Frequency of excursions.—During the convulsions there is a marked and more consistent decrease. The average is about 50 per cent, and in every case observations the excursions are longer than the normal in only two animals in the convulsions in twelve.

The average of the heart during the convulsions is a marked decrease. The frequency is reduced, and the amplitude of the excursions is longer than the normal in only two animals in the convulsions in twelve.

Frequency of excursions.—During the convulsions there is a marked and more consistent decrease. The average is about 50 per cent, and in every case observations the excursions are longer than the normal in only two animals in the convulsions in twelve.

Figure 1.—Two tracings of the heart of a frog. The upper tracing is taken from the left ventricle and the lower from the right ventricle. The tracings show the normal rhythm of the heart, with regular excursions of the heart muscle. The upper tracing shows a normal rhythm, with regular excursions of the heart muscle. The lower tracing shows a normal rhythm, with regular excursions of the heart muscle.

The average of the heart during the convulsions is a marked decrease. The frequency is reduced, and the amplitude of the excursions is longer than the normal in only two animals in the convulsions in twelve.

below normal in seven, above normal in four). The *systolic volume* tends to remain above normal (above in twelve, normal in six, below in four). The *diastolic volume* falls considerably below normal (below in fourteen, normal in nine, above in two).

Cardioplethysmograms. — Four compressions were made on a dog (C185), with divided vagi. The amplitude was increased somewhat during compression, generally decreased in the release fall. The diastolic volume increased somewhat more than the systolic during compression; in the release fall the diastolic and systolic volumes both fell slightly below normal.

Four compressions were also made on a dog (C182) with intact vagi. The excursions were unchanged during compression, diminished in the release fall. The diastolic and systolic volumes increased equally during compression. In the release both volumes fell below normal, the diastolic fall being greater than the systolic.

The plethysmographic method therefore gives essentially the same result as the lever methods.

From the cardiographic data it appears that *during the compressions* the excursions show an insignificant decrease, the diastolic volume being less increased than the systolic volume. This slight divergence is evidently due to the increased resistance which prevents complete systole. The efficiency of the cardiac muscle and of the valves is evidently not impaired.

In the release fall the diastolic volume falls below normal, while the systolic volume remains above normal; the excursions are thereby reduced.

The fall of the diastolic volume below the normal level could be due either to vasodilation or to lessened influx of blood into the heart. We have already shown that there is no vasodilation, whilst the filling of the empty vessels would naturally retard the return flow of blood to the heart. This must be one factor in the fall of blood pressure.

The simplest explanation of the failure of the systolic volume to return at once to the normal is probably that some time is required to adjust the tone of the cardiac muscle to the new pressure and volume. This must be a second important factor in the blood pressure fall.

Sudden death during aortic compression. — The experiment from which Fig. 4 was taken gave an interesting illustration of the sudden

death which is sometimes seen early in the compressions. The animal had been subjected to repeated compressions, alternating with intravenous injections of caffeine. When the total dosage of this drug

had reached 120 mg. per kilo, an aortic compression brought on *deffirm cordis* and gradual dilation of the ventricle, as shown in Fig. 3.



CONCLUSIONS.

I. *Effect of aortic compression on the vasomotor centre.* — Compression of the aorta causes normally a moderate constrictor response of the vasomotor centre.

This is not influenced by division of the vagi and is independent of the original level of blood pressure, unless this was below 70 mm. In the case of a severe aortic compression, with a marked

FIG. 3. The effect of aortic compression on the heart in a case of *deffirm cordis*. The curve shows the gradual dilation of the ventricle following aortic compression.

response, accompanied by reflex stimulation of the heart.

When the aorta is compressed, the reaction of the vasomotor centre is normally to the aorta.

When the compression is severe, the reaction of the vasomotor centre is to the aorta, and the response is moderate, during the compression, but is not normal.

When the aorta is compressed, the reaction of the vasomotor centre is to the aorta, and the response is moderate, during the compression, but is not normal. It is suggested that the reaction of the vasomotor centre is to the aorta, and the response is moderate, during the compression, but is not normal.

2. *Condition existing in the aorta during aortic compression.*

— The average *absolute rise* of blood pressure, if the vagi have been paralyzed, is practically constant for all levels of blood pressure between 40 and 120 mm. It is somewhat less below and above these levels. The average *percentile rise* therefore varies inversely to the level of pressure.

These phenomena are therefore parallel to those of the blood pressure response to sciatic stimulation at different levels. This furnishes additional evidence that the percentile rise is not a reliable index of vasomotor response.

If the vagi are active, the pressure does not rise as high, presumably on account of reflex vagus slowing of the heart.

Section of the vagi depressors after atropin does not modify the pressure response to aortic compression. This is further evidence that there is no effective depressor stimulation.

The rise of pressure is uniform in repeated compressions, provided that the other conditions are also uniform.

In prolonged compression the high pressure is maintained practically at a level, for some twenty minutes, after which time it may fall, at first slowly, then more rapidly.

The temporary fall of blood pressure which occurs on releasing the aorta is not due to vasomotor paralysis, central or peripheral.

III. Pulse pressure and cardiac tracings. — The membrane manometer tracings show, during the compression, increased pulse pressure, with the systolic pressure increased more than the diastolic pressure.

In the temporary fall of pressure following release of the aorta, the systolic pressure falls more than the diastolic pressure. The pulse pressure is diminished. This indicates lessened cardiac efficiency rather than vasodilation.

Cardiograms show that during the compression the amplitude of the excursions is but little diminished. The efficiency of the heart is therefore not seriously impaired. The systolic volume is increased relatively more than the diastolic volume. In the fall of release the systolic volume does not return quite to normal, the diastolic volume decreases below normal. This indicates lessened cardiac efficiency as the chief element in the fall. It is suggested that this is due to the time required for the tone of the cardiac muscle to adjust itself to the new condition of resistance, and to lessened return flow of blood to the heart.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH.—III. THE CONTRACTIONS OF THE EMPTY STOMACH INHIBITED REFLEXLY FROM THE MOUTH.

By A. J. CARLSON.

[From the Hull Physiological Laboratory of the University of Chicago.]

OUR man V. offers an exceptional opportunity for studying the relations of certain conscious states, particularly those associated with foods and with eating, on the activities of the empty stomach. The œsophagus is completely closed at the level of the upper end of the sternum, so that nothing can enter the stomach from the mouth. The swallowing mechanisms are normal, and the man can swallow and hold in the œsophageal pouch about 25 c.c. of material. The gustatory (and olfactory) sense is normal. The senses of thirst and hunger are normal. He masticates his food in the usual way, and the chewing processes are accompanied by the normal conscious states. The masticated food is placed in a syringe and introduced into the stomach through the fistula, which does not involve any pain or discomfort, and the man is adjusted to this condition, as this has been his method of feeding for the last sixteen years. Because of the ample size of the gastric fistula the man may sit down at the dinner-table, see, smell, taste, and chew his food in the usual manner up to the point of introducing the food through the fistula, while tracings are being taken of a tonus and the movements of the stomach, and records made of the secretion of gastric juice.

We know, particularly through the researches of Pawlow on dogs, that when appetite is present the sight, smell, or taste (especially taste) of palatable foods causes a reflex secretion of gastric juice, the so-called "psychic secretion." The efferent nerve fibres for this reflex reach the stomach through the vagi. The more recent work of Cannon and others has demonstrated that the tonus of the stomach musculature, at least of the empty stomach, is also primarily dependent on

efferent nervous impulses through the vagi. A certain degree of tonus is a prerequisite for peristalsis or contractions in the empty stomach. The suggestion is therefore obvious that the same stimuli which lead to psychic secretion of gastric juice may at the same time cause an augmentation of the tonus and the contractions of the stomach musculature. Cannon¹ has postulated such a "psychic tonus," but no evidence for it has been put on record.

It is now demonstrated that the sensation of hunger is caused by contractions of the empty stomach.² It is a universal experience that the sight or smell (or even the memory) of palatable foods may, apparently, induce hunger and appetite, or intensify these sensations if they are already present. The simplest explanation of this fact would seem to be that the smell or taste of palatable foods initiates or augments the stomach contractions, thus increasing the hunger sensation by increasing the intensity of the gastric stimulation. I expected to confirm this hypothesis when the present series of experiments were started, and was greatly surprised to find that the facts are the very opposite of those demanded by the hypothesis.

There are two sources of error in experiments of this character. In the first place, the periods of contraction of the empty stomach vary in intensity and duration, and the intervening periods of relative quiescence vary in length. The periods of quiescence may be interrupted by occasional contractions. This being the case, the initiation of stomach contractions simultaneously with tasting palatable food during quiescence of the stomach, for example, may be a mere coincident. An augmentation of the contractions seemingly due to tasting food during a contraction period may simply be the usual increase in strength of the stomach contraction during such period. In the same way, if tasting food towards the end of a contraction period should be followed by cessation of the stomach contractions, this apparent inhibition may be a coincident, the cessation of the contractions being "spontaneous" and not casually connected with the tasting of food. These difficulties were realized before the work was undertaken, as it was preceded by an extended survey of the "spontaneous" stomach movements when not interfered with experimentally. Because of the variability of the "spontaneous" stomach ac-

¹ CANNON: *The mechanical factors in digestion*, 1911, p. 200.

² CARLSON: *this Journal*, 1912, *xxi*, p. 175.

tivity, the individual tests must be repeated a great number of times, and little or no significance can be ascribed to *exceptional* results.

A source of error more serious, because not so readily controlled, lies in certain subjective states of an *inhibitory* character. Pawlow found that while the sight and smell of palatable foods ordinarily caused "psychic" secretion of gastric juice in dogs when hungry, if the dogs knew from past experience that they were not to be permitted to eat the food, the same stimuli caused no secretion. We may have analogous conditions in regard to the stomach tonus and movements. It is possible that, no matter how great the hunger or appetite in our man, the knowledge that the seeing, smelling, or tasting food was part of an experiment might initiate cerebral processes of an inhibitory character. This source of error has been controlled in two ways: (1) The mastication or tasting of the food was made part of his ordinary routine in preparing the food to be put into the stomach, and the man *knew* that as soon as the food was prepared it would be introduced into the stomach in the usual way; (2) Records were made of the presence or absence of the psychic secretion of gastric juice. If the tasting and chewing of the food start a copious flow of gastric juice, we can infer that the tasting and chewing do not give rise to cerebral processes of an inhibitory character.

1. **The inhibition of the contractions of the empty stomach by stimulation of the gustatory end organs in the mouth.** — The substances used for stimulation were sugar (solid and in solution), quinine in weak solution, sodium chloride (solid and in solution), weak solutions of acetic and of hydrochloric acids. Tests were made at all stages of activity of the empty stomach. The results were uniform and practically identical for the four kinds of stimuli employed. If the substances were used in sufficient concentrations to affect the stomach activity, the effects were inhibition of the tonus and the contractions. These inhibitory effects follow promptly on placing the substances in the mouth, and disappear, on the whole, very soon after removing the substances from the mouth and rinsing the mouth with warm water. Quinine and the acid produced the longest inhibitory after effects, probably because of the difficulty in completely removing these substances by rinsing the mouth with water.

This gustatory inhibition is, on the whole, proportional to the strength of the stimuli (*i. e.*, the concentration of the substance) and

varies inversely with the degree of the stomach activity. Thus a weak solution of acetic acid that produced distinct inhibition during the first stage of a period of hunger contraction when the individual

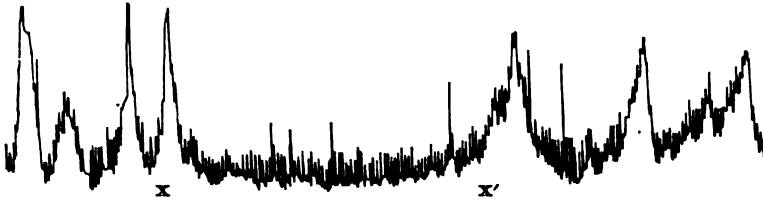


FIGURE 1. — One half the original size. At X a teaspoonful of sugar was put in the mouth. At X' the sugar was rinsed out with warm water. Showing inhibition of the stomach contractions by stimulation of end organs for the sense of sweetness.

contractions are relatively weak may have little or no effect when placed in the mouth during the tetanus stage of the contractions.

If the gustatory stimuli are weak and allowed to act in the mouth for five to fifteen minutes, the stomach "escapes" from the inhibition gradually. This is practically true of sweet (sugar). Moderate



FIGURE 2. — One half the original size. At X a teaspoonful of table salt was put in the mouth. At X' the salt was rinsed out with warm water. Showing inhibition of the stomach contractions.

strength of acids and quinine may hold the stomach in nearly complete inhibition up to fifteen minutes. The stimulating substances are, of course, gradually diluted by the secretion of saliva. The reader's attention is called to the typical tracings showing these inhibitions in Figs. 1-3.

Are these gustatory inhibitions primary and relatively simple

reflexes independent of the states of consciousness, or are they of the type of conditional reflexes, and therefore due to cerebral states of unpleasant effective tone? V. declared that the taste of quinine in any strength was always disagreeable. Strong acids and sodium chloride put in the mouth in solid form or in strong solution were

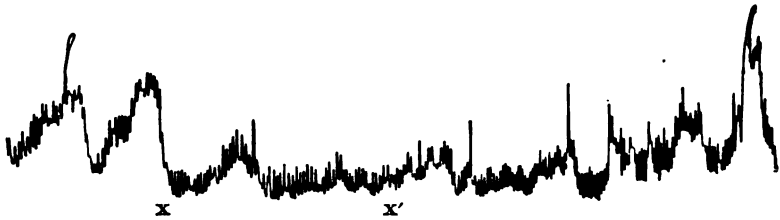


FIGURE 3. — One third the original size. At X 15 c.c. weak acetic acid was put in the mouth. At X' the mouth was rinsed with warm water. Showing inhibition of the stomach contractions.

also disagreeable. Weak acids and salt and sugar in any strength were not disagreeable. V. likes the taste of sugar, yet sugar in the mouth inhibits the movements of the empty stomach. I am inclined to the first hypothesis, or that these inhibitory phenomena are fundamental reflex inhibitions, associated with but not dependent on the states of consciousness. I propose to settle that point on decerebrated mammals and birds.

It may be stated that care was taken to avoid complications from swallowing movements in these gustatory stimulations. V. was instructed not to swallow under any circumstances, and these instructions were observed. When through salivation the quantity of fluid became too great to retain in the mouth, part of it was expectorated.

2. The inhibition of the tonus and the contractions of the empty stomach by chewing indifferent substances. — I have been unable to obtain any definite evidence of inhibition of the stomach movements by the movements of mastication when the mouth is empty. But chewing what may be called indifferent substances, such as paraffin, gum, or straw, produces distinct inhibition. Most of the experiments were made by chewing paraffin. To what extent paraffin is an "indifferent" substance to V. is difficult to determine. Most people can chew paraffin without any sensation of a disagreeable or unpleasant tone, or pleasant tone, either, for that matter. V. said he "did not care

for the paraffin," naturally. But I could get no evidence of a positive dislike for it. In the way of control of this point I frequently fixed V.'s attention on other matters during the chewing of paraffin, by engaging him in conversation, having him read items of interest (to him), or work out sums in arithmetic. In these cases the chewing of paraffin produced the typical inhibitory effects.

The chewing of indifferent substances produces, on the whole, less inhibition than do gustatory stimuli. The stomach "escapes" from the inhibition in a few minutes, even though the chewing is continued with uniform vigor. The chewing usually fails to produce any effects in the tetanus stage of the stomach activity. The tracing reproduced in Fig. 5 shows an exceptionally strong inhibition of this type.

Inasmuch as the masticatory movements do not cause inhibition if the mouth is empty, we may conclude that inhibition produced by chewing indifferent substances is initiated by mechanical stimulation of afferent nerve endings in the mouth.

3. *Inhibition of the tonus and the contractions of the empty stomach by chewing palatable foods when hunger and appetite are present.* — Tests were made with all food substances palatable to V. and during all stages of gastric tonus and contractions, which imply all degrees of hunger and appetite. But most of the experiments were made with meats in the form of stews, fricassees, or pot roasts, fried eggs, and crackers or bread soaked in milk, soups, or meat gravy. The results are uniform, without exception. Chewing or tasting palatable foods inhibits the tonus and the movements of the empty stomach. The inhibition is in evidence within a few seconds after placing the food in the mouth, and may or may not continue for some time after removing the food from the mouth and rinsing the mouth with warm water. The inhibition is least in evidence during the hunger tetanus. In fact, I am uncertain whether the chewing of palatable foods is able to materially affect the stomach in hunger tetanus. We have shown in previous communications that the stage of hunger tetanus is of variable length and ends abruptly in tonus relaxation and relative quiescence. This being the case, it is difficult to determine whether cessation of the hunger tetanus that follows — usually not very promptly — the placing of palatable food in the mouth is a "spontaneous" cessation, or due to inhibition from the mouth. The records show, however, that so far as the stimuli in the mouth affect the pro-

cesses of the hunger tetanus, the influence is in the direction of inhibition. A tracing showing inhibition of the hunger contractions on chewing meat is reproduced in Fig. 6.

The inhibition of the motor activity of the stomach by chewing palatable foods does not appear to have any after effects in the nature of increased tonus or contractions. Some of the tracings do suggest a motor after effect, but I am inclined to interpret them in a different way. These effects are obtained only when the tests are made during the relative quiescence of the stomach or at the beginning of a contraction period ("thirty-seconds rhythm"). Moreover, these results were not always secured even during these periods. It would therefore seem that these apparent augmentary after effects represent the "spontaneous" initiation of a contraction period, or the gradual increase in the magnitude of the contractions characteristic of the periods of the thirty-seconds rhythm.

4. The factors involved in the inhibition of the contractions of the empty stomach by palatable foods in the mouth. — Boldireff³ has reported that the contractions of the empty stomach in the dog cease during the periods of "spontaneous" secretion of gastric juice. We know that tasting or chewing palatable foods leads to reflex or "psychic" secretion of gastric juice in mammals (including man). May not the inhibition described above be an indirect one due to the secretion of gastric juice, rather than a reflex inhibition of more direct character? This question has been investigated and settled. A rapid secretion of gastric juice is associated with cessation, partial or complete, of the stomach contractions in V. In a later paper facts will be reported which seem to prove that this is due, not to the processes of secretion, as such, but to acid stimulation of nerve endings in the mucosa. When the chewing or tasting of palatable foods leads to copious secretion of gastric juice, this gastric juice is one factor in the accompanying inhibition of the stomach movements.

We know, from Pawlow's work on dogs, that the latent period of the "psychic" secretion is about five minutes. The latent period of the "psychic" secretion in man is also relatively long. I have not yet determined the average duration in V., except that in no case is it less than three minutes. The inhibition of stomach tonus and movements follows within a few seconds after placing the food in the mouth.

³ BOLDIREFF: Archives des sciences biologiques, 1905, xi, p. 1.

Hence it is not an acid inhibition from the stomach. The same thing can be shown by some instances when the tasting or chewing of the food produces only a scanty secretion of gastric juice. The inhibition appears in the normal way, and the contractions reappear on removing the food from the mouth despite the slow secretion of gastric juice.

It seems that a certain quantity of gastric juice must accumulate in the stomach or the free hydrochloric acid in the stomach must reach

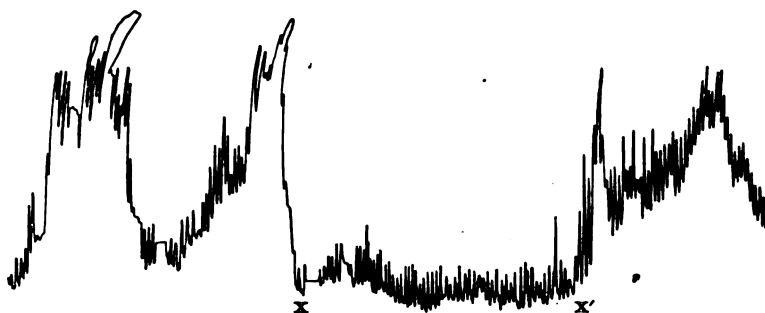


FIGURE 4. — One half the original size. $X-X'$, vigorous chewing of paraffin. Showing inhibition of the hunger contractions of the stomach by chewing indifferent substances.

a certain concentration before the acid inhibition takes place. Thus, if the period of chewing or tasting the palatable food is short (four to six minutes), the stomach contractions may reappear at the end of the stimulation in the mouth, and shortly afterwards again be inhibited by the acid gastric juice. This inhibition continues during the phase of rapid "psychic" secretion. When the psychic secretion is more copious, the reflex inhibition from the mouth merges into the acid inhibition from the stomach. Tracings illustrating this phenomenon will be published in a later communication.

We have discussed the inhibition following the tasting or chewing of palatable foods when hungry under a separate head, but as regards that part of it which constitutes a reflex effect from the mouth we may not be dealing with new afferent mechanisms. Since stimulation of the organs of taste, and chewing indifferent substances cause inhibition, the inhibition due to tasting and chewing palatable foods may result from a combination of these two factors.

5. The inhibition of the tonus and the contractions of the empty stomach

by swallowing movements. — It has been shown by Cannon and Lieb ⁴ for the dog that the movements of swallowing lead to a temporary inhibition of the tonus of the stomach. This inhibition is designated the "receptive relaxation" of the stomach. This inhibition is readily demonstrated in man. When V. makes repeated swallowing move-

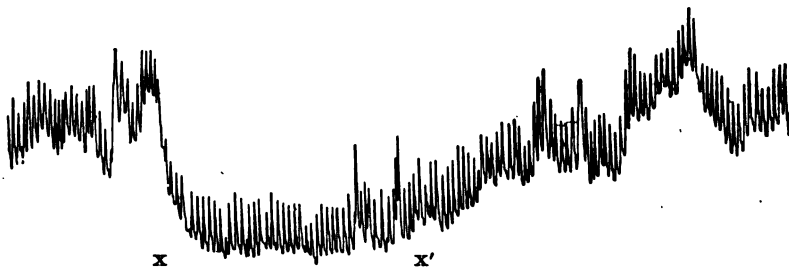


FIGURE 5. — Four sevenths the original size. $X-X'$, repeated swallowing of small quantities of saliva (the swallowed saliva does not reach the stomach). Showing inhibition of the tonus and contractions of the empty stomach.

ments with only enough saliva in the mouth to initiate the swallowing reflex, a prompt but transitory inhibition of gastric tonus and contractions is produced. The reader will recall that the swallowed saliva does not reach the stomach, but collects in the œsophagus pouch. Complete inhibition of the stomach contractions was never secured through the swallowing act, and when the stomach is in the condition of hunger tetanus, or in very strong and rapid contractions bordering on tetanus, the swallowing movements seem to have no effect on the stomach. The inhibition of the stomach tonus due to the act of swallowing is most readily demonstrated at the beginning of a period of hunger contractions. A tracing illustrating this inhibition is reproduced in Fig. 5.

6. The relation of the reflex inhibition of the tonus and the movements of the empty stomach from the mouth to the sensations of hunger. — In the second communication of these studies on Mr. V., it was demonstrated that the stomach contractions give rise to the sensation of hunger, or the hunger "pangs." This being the case, we should expect that the stimulation of the gustatory end organs in the mouth, the chewing of indifferent substances, and the tasting and chewing of palatable foods would abolish the sensations of hunger to the same degree that these measures inhibit the stomach contractions. And

⁴ CANNON and LIEB: this Journal, 1911, xxvii, p. xiii.

that is the fact. The inhibition of the stomach activity and the cessation of the hunger pains run parallel. If the inhibition of the contractions is complete, V. does not touch the hunger signals. If the inhibition is incomplete, V. presses Key No. 1 (weak hunger) or Key No. 2 (moderately strong hunger), as the case may be. In almost all

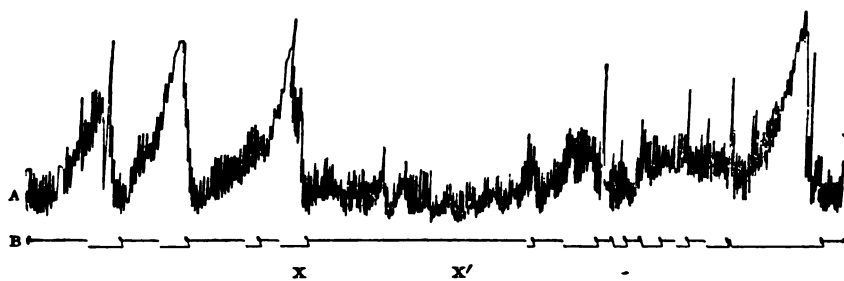


FIGURE 6. — One third the original size. A, stomach contractions; B, hunger signal; X-X', chewing meat (palatable). Showing inhibition of the contractions of the empty stomach and the parallel cessation of the hunger pangs.

of the experiments in this series records of the hunger sensations were taken simultaneously with those of the hunger contractions. A typical record showing this parallel between the stomach inhibition and the cessation of hunger sensations is reproduced in Fig. 6.

7. *Discussion of the results.* — The detailed mechanism of the inhibition described in the foregoing pages remains to be worked out. The nerves of taste and at least some of those of general sense in the mouth constitute the afferent path. The actual inhibition is brought about either by a complete reflex through the inhibitory fibres in the splanchnic nerves and the vagi, or else the inhibition of the vagus tonus through action on the lower brain centres is the main factor.

Pawlow designates the secretion of gastric juice produced by the sight, taste, or smell of palatable foods when hungry as "psychic" secretion, thereby implying that cerebral or conscious processes constitute a necessary link in the reflex. We might on the same basis designate the inhibition of stomach movements by the taste of palatable foods as "psychic" inhibition. But I doubt the correctness of this position. I have so far been unable to demonstrate any influence on the stomach motor activity from the sight or smell of palatable foods in V. In dogs the sight or smell of food may either increase or inhibit the movements of the empty stomach. The absence of this

effect in V. is probably due to a rather stolid disposition, those stimuli not producing the proper cerebral states, or at least not of sufficient intensity. The effect on the stomach movements of the sight and smell of foods (when in evidence) is a reflex probably involving psychic elements in the form of memory processes from previous experience with food. But I am inclined to believe that the inhibitions of the stomach activity from the oral cavity described in this paper are primary or fundamental reflexes not dependent on memory processes from the individual's experiences. The hypothesis can be established — or refuted — on decerebrated animals. It may be noted in this connection that similar inhibitions of the stomach from the mouth seem to obtain in the dog, but the difficulty of controlling salivation and swallowing in the dog renders the data less conclusive than on man. Comparative studies will in all probability demonstrate the presence of similar inhibitory reflexes in all groups of vertebrates. A consideration of the biological significance of these inhibitions in advance of such comparative investigations is, of course, premature. Possibly they are to be designated as "receptive inhibitions" analogous to the "receptive relaxation" of the stomach in swallowing, as palatable foods in the mouth are normally destined for the stomach. These inhibitions may also be said to conform to the "law of the intestine" of Bayliss and Starling, to the extent that the inhibition appears caudad to the point of stimulation. There is, of course, a fundamental difference in the nervous mechanism involved.

It is a common experience that *appetite* is augmented by tasting palatable foods. We have shown in this paper that hunger is inhibited by tasting or chewing palatable foods. It would seem that hunger has reached its biological end as a motor stimulus when the foods reach the mouth, and at that stage appetite takes the place of hunger as the guide to the quantity of foods to be ingested.

The influence of the tasting and chewing palatable foods and of stimulation of the gustatory end organs on the movements of the stomach in digestion has not yet been determined. The movements of the filled stomach are probably not inhibited. The fact that normal gastric juice inhibits the movements of the empty stomach but does not inhibit the stomach movements in digestion seems to indicate that the motor activities of the empty and of the filled stomach involve, in part, different mechanisms.

VARIATIONS IN IRRITABILITY OF THE REFLEX ARC. —
I. VARIATIONS UNDER ASPHYXIAL CONDITIONS,
WITH BLOOD-GAS DETERMINATIONS.

By E. L. PORTER.

[From the Laboratory of Physiology in the Harvard Medical School.]

INTRODUCTION.

IF reflex-arc conduction be compared with conduction in the nerve trunk, there appear important differences between the two processes. For example, the reflex arc fatigues more easily, it is much more susceptible to asphyxial conditions, and it succumbs more easily to anæsthetics.¹ The reflex is thus a mechanism which varies in its efficiency as compared with nerve trunks with their relatively greater stability of function. The limits of variation are wide, extending on the one hand from a condition of optimum efficiency in the normal animal down to a complete loss of irritability in an animal under deep narcosis. When mechanisms of such importance to the organism as reflexes exhibit such wide variations in efficiency, it becomes of considerable importance to obtain as extensive and accurate knowledge as possible of the conditions under which such variations occur.

The present paper is a report of part of a research on this problem undertaken at the suggestion of Dr. W. B. Cannon. The plan was to select some very simple reflex in the spinal animal, measure accurately the threshold value of the stimulus necessary to elicit it, and then follow carefully the alterations in the value of this threshold during various experimental procedures, these procedures being such as might be expected to influence reflex activity. A research of this kind is not only of value as a quantitative study of one of the great "integrative" mechanisms of the body, just as studies on variations

¹ SHERRINGTON: The integrative action of the nervous system, New York, 1906, p. 7.

in the efficiency of the circulatory system have value, but it is also of interest from another point of view. The conception of the synapse has become firmly entrenched in physiology and, for a mechanism as yet hypothetical, its duties have been made heavy. The chief differences between reflex-arc conduction and nerve-trunk conduction are ascribed to its presence. Any study of variations in reflex conduction is then at bottom a study of conditions under which the synapse will or will not permit impulses to pass across it, and hence involves a fundamental point in the physiology of the nervous system.

Quantitative studies on reflexes have been comparatively few, in large measure due probably to the difficulty of measuring the stimuli accurately. Bergman,² in a study of the reflex activity of a frog after the circulation had been interrupted, used for stimulation a du Bois-Reymond inductorium graduated according to the Fick method. The skin over the patella, or the central end of the sciatic nerve, was stimulated to cause reflex withdrawal of the leg. The alterations in the condition of the reflex were followed for several hours in some cases, and results obtained and curves plotted which resemble those to be reported in this paper. Baglioni³ used a reflex preparation consisting of the frog's spinal cord which had been laid bare and isolated from the rest of the central nervous system, but left connected with the hind leg through the sciatic nerve. This whole preparation was placed in various gaseous or liquid mixtures, and the conditions noted under which reflex irritability disappeared when the skin of the foot was stimulated. No account was taken of alterations in irritability, however, other than complete loss of reflex movement. Pari⁴ studied the relation between the strength of stimulus and the height of reflex contraction. The strength of stimulus was given in terms of the distance of the secondary coil from complete superposition over the primary coil. Van Reekum,⁵ working under Zwaardemaker, used condenser discharges as a means of stimulation in a research on the threshold value of reflex movement of the foot in the spinal frog. This permits the value of the stimulus to be stated in terms of energy in

² BERGMAN: *Skandinavisches Archiv für Physiologie*, 1897, vii, p. 198.

³ BAGLIONI: *Zeitschrift für allgemeine Physiologie*, 1904, iv, p. 384.

⁴ PARI: *Archives italiennes de biologie*, 1904, xlii, p. 109.

⁵ VAN REEKUM: *Quantitative Onderzoekingen over Reflexen*. Doctor's dissertation. Utrecht, 1906.

the centimetre-gram-second system. It is an accurate method if the conditions of experimentation are kept uniform. Porter,⁶ Sherrington,⁷ and Fröhlich⁸ have also made quantitative studies on reflexes, stating the value of the stimuli in Kronecker units.

The objection which can be raised against these and similar researches as quantitative studies is that the results are accurately comparable only for a single experiment. In the present research the attempt has been made to overcome this objection by the use of the Martin method⁹ of measuring the value of the stimulus, by which, if rigorously applied, correction is made for all the varying factors in stimulation with induction shocks. When the method is applied thus thoroughly, the results are stated in β units. This requires a considerable amount of calculation for each determination of β . I have not done this, since the number of determinations of reflex threshold was too large to make such calculation feasible, and the purpose of the investigation was sufficiently well served without. The results have therefore been stated in Z units.¹⁰

METHOD FOR THE STUDY OF VARIATIONS IN IRRITABILITY.

The method of preparing the animal and of measuring the value of the stimulus has been described in a previous paper.¹¹ Briefly it consisted in applying an electrode to the tibial nerve of the spinal cat and measuring the value of the stimulus necessary to elicit the flexion reflex. An electrode was also applied to the dorsal interosseous branch of the radial nerve on the fore-limb, stimulation of which caused extension at the wrist, a movement which was neuromuscular, not reflex. This served as a control, indicating how much the alteration in threshold could be ascribed to central changes and how much to neuromuscular. When an animal had been thus prepared, the thresh-

⁶ PORTER, W. T.: this Journal, 1910, xlvii, p. 276.

⁷ SHERRINGTON: Journal of physiology, 1910, xl, p. 28.

⁸ FRÖHLICH: Zeitschrift für allgemeine Physiologie, 1909-1910, x, p. 396.

⁹ MARTIN: this Journal, 1911, xxviii, p. 49, and earlier papers. See also MARTIN: The measurement of induction shocks, New York, 1912.

¹⁰ For the amount of error involved in the use of Z units see MARTIN: Measurement of induction shocks, p. 80.

¹¹ PORTER, E. L.: this Journal, 1912, xxxi, p. 141.

old of the flexion reflex was determined at short intervals while the animal was subjected to those experimental conditions by which it was proposed to raise or lower the threshold. Parallel readings were made on the neuromuscular mechanism. At the conclusion of the experiment the results were plotted in the form of curves with time in

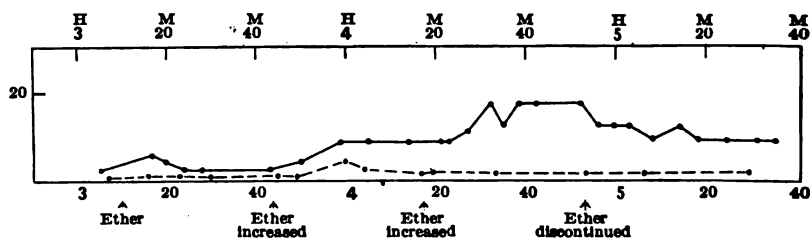


FIGURE 1.—Behavior of reflex and nerve-muscle preparations under ether. Method described in the text. Ordinates represent values of Z, abscissæ represent time in hours and minutes since the beginning of the experiment. Continuous line, reflex record; broken line, nerve-muscle record.

hours and minutes as abscissæ and values of the stimuli in Z units as ordinates. An example of such a record is shown in Fig. 1, in which ether in varying amounts was administered by shunting through an ether bottle a part of the air for artificial respiration. As the ether was administered a stronger stimulus was required to elicit the reflex, and the curve (continuous line) rose. The irritability of the nerve-muscle preparation (broken line) remained practically unaltered. Removal of the ether was followed by a lowering in threshold, although not to the original level.

A complete graphic record of this sort serves a purpose in the study of the functional efficiency of the cord similar to that served by a graphic record of blood pressure in a study of the circulation. Such quantitative records for mammals have never been published, so far as the writer is aware.

VARIATIONS OF REFLEX THRESHOLD UNDER ASPHYXIAL CONDITIONS.

The present paper is concerned only with the variations under asphyxial conditions, with a consideration of the blood-gas content at the moment reflex irritability was lost.

The literature on the relation of asphyxia to the nervous system

has dealt very largely with the activity of the respiratory centre and the nature of the stimulus to that centre. Whether this centre acts toward the gases of the blood as do the cells of the cord is an unsettled question. One point, however, has been very evident to all recent investigators of the subject, namely, that account must always be taken of the two factors of asphyxia: diminished oxygen supply, and excess of carbon dioxide — factors which may or may not vary together. Although the present research involved only the cord and not the respiratory centre, it was necessary, of course, to take the two factors into account. Verworn¹² has emphasized their importance in one of his researches. In his method a frog is poisoned with strychnine, the gastrocnemius muscle arranged to write its record on a drum, and the sciatic nerve blocked by local ether narcosis to prevent fatigue of the muscle. After convulsions have taken place for some time, the block is removed, and the gastrocnemius allowed to write its record on a drum. When the skin is stimulated, contractions occur in groups instead of continuously, showing, as Verworn thinks, that the spinal nerve centres are fatigued. The heart soon ceases to beat, and the gastrocnemius contractions no longer appear. The spinal cord is in a condition of paralysis. The most important of his observations and conclusions in regard to this condition are the following:

(1) The paralysis is partially due to the accumulation of metabolic products in the cord, for temporary recovery can be brought about by irrigating the blood vessels of the frog with oxygen-free saline.

(2) The conclusion in (1) is rendered still more probable by an experiment with one of the metabolic products of the organism, namely, carbon dioxide. A strychninized frog is placed in an atmosphere of 80 per cent carbon dioxide and 20 per cent oxygen. Reflex activity is very quickly lost, generally before the appearance of strychnine convulsions. If the narcotized animal is now placed in pure hydrogen, the convulsions soon appear.

(3) The paralysis is also due in part to lack of oxygen. This was shown as follows: A strychninized frog was stimulated until no further reflex contractions occurred; the blood vessels were then irrigated with oxygen-free salt solution and the reflexes appeared again as in (1). The frog was stimulated still further until in spite of the irrigation no further reflex contractions could be evoked. If now oxy-

¹² VERWORN: *Archiv für Physiologie*, 1900, Supplement-Band, p. 152.

generated salt solution was used for irrigation, there was a temporary return of reflex activity.

The blood-gas content under adequate artificial respiration. — Since it was planned to make determinations of the gas content of the blood under asphyxial conditions, it became necessary for purposes of comparison to know the gas content under conditions which were not asphyxial. Experimental difficulties made impracticable the attempt to secure such data for the intact animal, but numerous determinations have been made on spinal cats at normal temperature and with the artificial respiration supplying air in amounts which experience has shown are amply sufficient to maintain the irritability of the reflex unchanged for long periods. The analyses were made by the Barcroft-Haldane apparatus,¹³ of which a modified form was used, modelled on that used by Henderson.¹⁴ The determinations will be summarized here to supply standards of comparison when the experimental values are referred to. The values thus obtained for twenty-two cases for oxygen and twenty-five for carbon dioxide in volumes per cent are shown in Table I. The blood was drawn from the carotid artery.

According to these results the blood of the spinal cat under adequate artificial respiration contains an average of 11.5 vols. per cent oxygen and 32.6 vols. per cent carbon dioxide.

Variations in irritability under simple asphyxia. — The first asphyxial condition studied was that brought about by the simple expedient of cutting off the artificial respiration. The animal was prepared in the usual way and readings of reflex threshold made at short intervals. If now the air supply was cut off, there ensued in a few minutes, generally within two or three, the well-known asphyxial convulsions, and shortly afterward the reflex disappeared, or, to be more exact, it could not be elicited with the strongest stimulus convenient to apply with the apparatus used, that is, a stimulus of 600 Z units in value. This is approximately one hundred times the value of the normal stimulus of 5.2 Z units.¹⁵ Upon again supplying air the reflex promptly returned. When the curve for such an experiment was plotted, it was invariably found to have the form shown in Fig. 2. The significant feature of the curve, as contrasted with that shown in Fig.

¹³ BARCROFT-HALDANE: *Journal of physiology*, 1902, xxviii, p. 232.

¹⁴ YANDELL HENDERSON: *this Journal*, 1910, xxv, p. 326.

¹⁵ PORTER, E. L.: *Loc. cit.*, p. 148.

r, is the suddenness with which the curve rises to great height a few moments after the air is cut off. In Fig. 1 the administration of ether led to a rise in threshold, but it was gradual, and at its highest point

TABLE I.

CARBON DIOXIDE AND OXYGEN IN THE BLOOD OF THE SPINAL CAT UNDER ADEQUATE ARTIFICIAL RESPIRATION.

O ₂ Vols. per cent.	CO ₂ Vols. per cent.	O ₂ Vols. per cent.	CO ₂ Vols. per cent.
6.7	34.8	16.5	30.0
4.7	43.0	8.8	32.7
8.0	56.0	10.1	18.5
9.2	32.0	. . .	33.1
9.5	48.8	. . .	30.0
9.2	53.0	. . .	35.3
15.5	31.4	14.8	24.1
15.4	36.0	13.3	27.0
10.5	31.7	10.9	25.1
11.0	32.1	7.5	22.0
14.5	27.9	5.7	22.1
16.8	27.3	16.5	26.0
17.1	34.6
Average		11.5	32.6

the threshold was only about three times as high as at the beginning of the experiment. The shape of the curve in Fig. 2 is very similar to those quoted by Bergman¹⁶ for frogs in which the circulation was interrupted. In the case of the frogs, however, the reflex was not lost for from thirty to fifty minutes after the circulation had been cut off, as contrasted with two or three minutes for the spinal cat in asphyxia.

¹⁶ BERGMAN: *Loc. cit.*

Blood-gas content in simple asphyxia.—The shape of the asphyxial curve suggested that the blood-gas content of the animal at the time of the sudden rise in threshold might be uniform and significant.

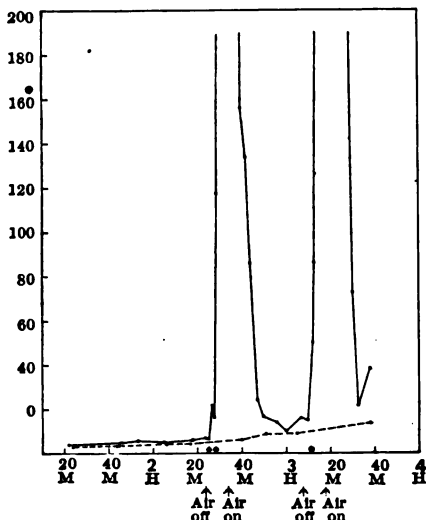


FIGURE 2.—Behavior of the reflex under asphyxia. Artificial respiration discontinued between 2.23 and 2.31 and between 3.09 and 3.18 as indicated by the arrows. Ordinates, values of Z ; abscissae, elapsed time in hours and minutes. Continuous line, reflex; broken line, nerve-muscle preparation. Asterisks indicate convulsions.

There might, for instance, be a certain amount of carbon dioxide which was fatal to the integrity of the reflex arc. And, since the character of the curve seemed to be such an accurate index to the condition of the reflex, it was thought that it might be possible to withdraw the blood within a few seconds of the time when the reflex went out of function and determine from the sample the significant or critical blood-gas content at that time. Nineteen such analyses have been made on twelve cats. The procedure has been to follow the threshold of the reflex rather closely with frequent readings, and then, with the blood syringe in readiness and a cannula in the carotid, to remove the rubber tube of the artificial respiration system from the tracheal

cannula, thus cutting off the air supply. The threshold of the reflex is now followed carefully with perhaps two readings a minute, as asphyxia develops. When the threshold rises suddenly to a great height, the syringe is immediately applied to the rubber tube connected with the carotid cannula and a sample of blood drawn. It has been possible to make the drawing of the blood coincide very accurately with the rise in the threshold of the reflex. The air tube is immediately replaced on the tracheal cannula, and the sample of blood then analyzed. The values which have thus been obtained are shown in Table II.

From the results it appears that at the time the reflex is lost the average blood-gas content is, oxygen 6.4 vols. per cent and carbon

dioxide 30.6 vols. per cent. The oxygen has dropped to a value which is approximately 56 per cent of that under artificial respiration. The carbon dioxide, instead of showing an increase over that for artificial respiration, shows a decrease in the average amounting to 6 per cent. This difference compared with variations in the several cases is not considered significant. The results point strongly to the conclusion that the loss of the reflex was due to the diminished oxygen supply and not to a change in the amount of carbon dioxide.

TABLE II.

BLOOD-GAS CONTENT IN THE SPINAL CAT AT THE MOMENT OF DISAPPEARANCE OF THE FLEXION REFLEX IN CONSEQUENCE OF STOPPING THE ARTIFICIAL RESPIRATION.

O ₂ Vols. per cent.	CO ₂ Vols. per cent.	O ₂ Vols. per cent.	CO ₂ Vols. per cent.
4.55	33.3	6.5	23.0
11.20	29.1	3.7	27.0
5.00	33.7	9.2	34.1
11.5	24.0	7.0	38.4
5.2	29.7	7.1	37.4
0.2	26.0	5.6	34.9
Average		6.4	30.6

Variations in irritability under diminished oxygen without excess of carbon dioxide. — The next problem which presented itself was to separate the two factors of asphyxia mentioned above, namely, diminished oxygen and excess of carbon dioxide, and find what the independent effect of each was on the reflex activities of the spinal animal. The problem of diminished oxygen was first attacked.

A condition of oxygen lack without excess of carbon dioxide can be brought about easily, of course, by using for artificial respiration nitrogen or hydrogen in place of air. The inert gas washes out the carbon dioxide but furnishes no oxygen. No nitrogen or hydrogen supply for this purpose being easily available in the laboratory at the time this work was being done, I tried the following method, which proved entirely satisfactory.

A tall specimen jar (Fig. 3, *a*), about 8 cm. in diameter, was closed by a two-holed rubber stopper bearing short pieces of glass tubing.

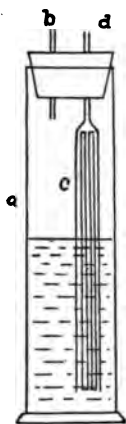


FIGURE 3. — Arrangement for securing diminished oxygen without excess of carbon dioxide. Explained in the text.

To one glass tube (*d*) was attached, inside the jar, a piece of wider glass tubing (*c*) reaching nearly to the bottom of the jar. This large tube was filled with lengths of smaller tubing to increase the surface. The jar was half filled with a strong solution of potassium hydrate. The tip-bucket of the artificial respiration scheme¹⁷ was so arranged that if tipped in one direction air would be driven into the jar through tube *b*, but if tipped in the other the air in the jar would be free to escape back through tube *b* and a vent in the tip-bucket into the room. The tube *d* was connected with the tracheal cannula. When air entered *b*, it drove the solution in the jar downward, but at the same time raised the level of the liquid in the tube *c*, forcing the air above it into the animal's lungs. When the bucket tipped to the opposite side, the level of the solution fell once more in *d*, the air was withdrawn from the lungs back into the tube, while the air in the jar escaped to the room through the vent in the tip-bucket in sufficient quantity to allow the two portions of the solution to come to a level. The carbon dioxide from the lungs was absorbed by the potassium hydrate solution which covered the inner walls of tube *c* and the glass tubing inside. The oxygen was soon exhausted from the small amount of air driven into and out of the lungs, and asphyxial phenomena promptly appeared in the form of convulsive movements, followed by loss of reflex activity.

A typical curve of reflex irritability obtained by the method just described is shown in Fig. 4. Its resemblance to that obtained in simple asphyxia (Fig. 2) is obvious.

Blood gases under diminished oxygen and no excess of carbon dioxide. — The blood-gas content was determined as before by taking a sample of blood at the moment of disappearance of the reflex. The values

¹⁷ An air supply under constant pressure regulated by the tip-bucket supplied by the Harvard Apparatus Company, constituted the mechanism for artificial respiration.

obtained from twelve analyses are given in Table III. The results show that when there is diminished oxygen but no excess of carbon dioxide the reflex disappears with a blood-gas content averaging 4.5 vols. per cent of oxygen and 27.3 vols. per cent of carbon dioxide. The analyses demonstrate that the device illustrated in Fig.

TABLE III.

BLOOD-GAS CONTENT AT THE MOMENT OF DISAPPEARANCE OF THE FLEXION REFLEX IN CONSEQUENCE OF DIMINISHED OXYGEN, BUT WITHOUT EXCESS OF CARBON DIOXIDE.

O ₂ Vols. per cent.	CO ₂ Vols. per cent.	O ₂ Vols. per cent.	CO ₂ Vols. per cent.
11.6	30.5	1.1	31.2
8.4	35.2	3.2	23.6
1.8	23.4	0.6	22.9
2.9	33.7	12.4	22.7
8.9	30.0	1.4	24.0
0.0	29.5	1.2	21.3
Average		4.5	27.3

3 worked successfully; the oxygen of the blood had been reduced to considerably less than half the value under artificial respiration (11.5 vols. per cent, Table I), and the carbon dioxide not only was not increased in amount but averaged 5.3 vols. per cent less than the artificial respiration value (32.6 vols. per cent).

Variations in irritability under excess of carbon dioxide with sufficient oxygen.— Volhard ¹⁸ found that if a very gentle stream of oxygen were blown into the trachea of a spinal animal, or one which had been curarized, the blood was amply oxygenated, but that under these circumstances the carbon dioxide accumulated in the blood. It can be made to accumulate faster by blowing into the trachea a mixture of carbon dioxide and oxygen. This is the method which has been used in this research to study the effect on the flexion reflex of excess of carbon dioxide with sufficient oxygen. If a mixture of 50 per cent carbon dioxide and

¹⁸ VOLHARD: Münchener medizinische Wochenschrift, 1908, lv, p. 209.

50 per cent oxygen be used, the effect on the reflex is as shown in Fig. 5, which is a typical case. These curves bear a general resemblance to those obtained under asphyxia or diminished oxygen (Figs.

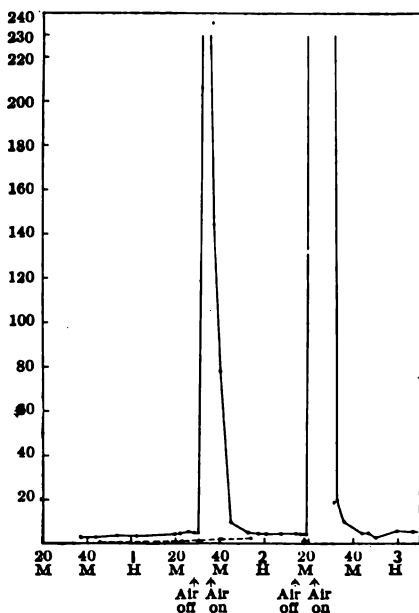


FIGURE 4.—Behavior of the reflex under diminished oxygen supply but without excess of carbon dioxide. Diminished oxygen between 1.28 and 1.34 and between 2.16 and 2.22 as indicated by the arrows. Method described in the text. Ordinates, values of Z; abscissæ, time in hours and minutes. Continuous line, reflex; broken line, nerve-muscle preparation. Asterisks indicate convulsions.

administration of a mixture of 50 per cent oxygen and 50 per cent carbon dioxide, the reflex disappeared when the blood contained an average of 23.8 vols. per cent oxygen and 47.2 vols. per cent carbon dioxide. This is an excess over the average for artificial respiration with air of 14.6 vols. per cent carbon dioxide and 12.3 vols. per cent oxygen. This excess of oxygen was not intentional. It seemed difficult to so regulate the supply as to maintain the oxygen at the level obtained under respiration with air.

2 and 4), but with these differences, that there is a preliminary rise in the threshold before the reflex disappears, and that the reflex does not disappear promptly. A delay of fifteen to twenty minutes occurs after beginning the administration of carbon dioxide and before the disappearance, as compared with three to four minutes after cutting off the oxygen supply. The threshold eventually rises, however, with an abruptness nearly as marked as in the case of diminished oxygen or simple asphyxia.

Blood-gas content under moderate administration of carbon dioxide.—Determinations of the blood-gas content were made as before at the moment the reflex disappeared, and the results of the ten analyses made are shown in Table IV. They show that with excess of carbon dioxide but with the oxygen not diminished, when this condition was secured by the

Results with administration of low percentages of carbon dioxide. — The relation between administration of carbon dioxide and loss of the reflex is not so simple as these curves and figures just given would indicate, for if the carbon dioxide is given in less proportions, say 30 per cent carbon dioxide and 70 per cent oxygen, or a smaller proportion of

TABLE IV.

BLOOD-GASES DETERMINED AT THE TIME WHEN THE FLEXION REFLEX DISAPPEARED IN CONSEQUENCE OF THE ADMINISTRATION OF 50 PER CENT OXYGEN AND 50 PER CENT CARBON DIOXIDE BY THE VOLHARD METHOD.

O ₂ Vols. per cent.	CO ₂ Vols. per cent.	O ₂ Vols. per cent.	CO ₂ Vols. per cent.
16.1	44.6	25.1	48.3
28.3	51.5	21.1	40.8
29.4	50.1	15.8	42.9
32.8	41.8	14.4	46.6
22.3	48.9	33.3	56.1
Average		23.8	47.2

carbon dioxide, very different results are obtained. Figs. 6 and 7 show curves obtained under such conditions. The threshold of the reflex did not rise in Fig. 7 with any suddenness until thirty-one minutes after beginning respiration with the carbon dioxide mixture. The carbon dioxide in the blood had at 3.55 reached a value of 63.4 vols. per cent. This is 16.2 vols. per cent above the value given in Table IV.

In the experiment represented in Fig. 6 the mixture of gases at first administered was 5 per cent carbon dioxide and 95 per cent oxygen. This was gradually increased to 30 per cent carbon dioxide and 70 per cent oxygen. At 5.37, soon after the sharp rise in threshold, there were in the blood 31.3 vols. per cent of oxygen and 82.6 vols. per cent carbon dioxide. This is nearly twice the amount of carbon dioxide which was fatal to the reflex when a mixture of 50 per cent oxygen and 50 per cent carbon dioxide was used for respiration. In these cases of excessive amounts of carbon dioxide in the blood before the reflex dis-

appeared, there had obviously been some sort of adaptation either by the nervous system or in the blood. Henderson's conclusions¹⁹ would indicate that this may occur mainly in the blood.

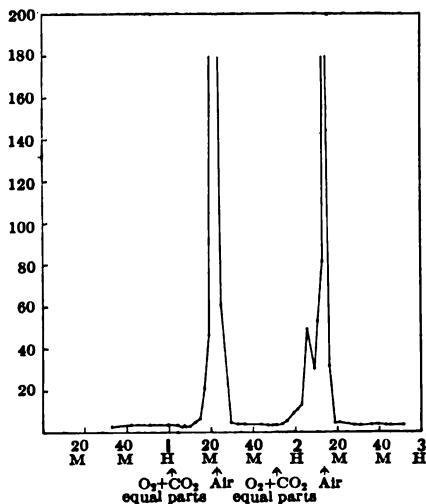


FIGURE 5.—Behavior of the reflex under excess of carbon dioxide and undiminished oxygen, demonstrating the abrupt rise in threshold when high percentages of carbon dioxide are administered. Between 1.01 and 1.23 and between 1.47 and 2.15 carbon dioxide and oxygen in equal parts were administered by the Volhard method. Ordinates, values of Z; abscissæ, elapsed time in hours and minutes.

The amount of carbon dioxide in the blood apparently steadily increases under moderately slow administration of this gas, and the reflex may or may not disappear. In the case of diminished oxygen supply the disappearance of the reflex is roughly an indication that a certain less amount of oxygen is present in the blood, but it is not similarly an indication of the increased amount of carbon dioxide in the blood when that gas is in excess and the oxygen not diminished. For the amount of carbon dioxide when the reflex disappears will be greater or less depending on the rapidity with which it was administered. Indeed, unless the carbon dioxide be supplied in a sufficiently large proportion, it may be impossible to raise the threshold of the re-

flex very high. Fig. 7 illustrates this. Here the threshold never went higher than 117 Z units, and reached this height only after fifty-three minutes of administration of a mixture of 30 per cent carbon dioxide and 70 per cent oxygen, although the blood contained at this time 63.4 vols. per cent of carbon dioxide.

RELATION OF BLOOD PRESSURE TO DIMINISHED IRRITABILITY.

In drawing the blood for analysis while the animal was being subjected to any of the asphyxial conditions described above, it was evident in many cases that the heart was in poor condition, that is,

was beating weakly or irregularly. Many times just at the moment when the blood was about to be drawn the analysis had to be abandoned because the heart ceased to beat. These occurrences suggested that the blood-gas content might be of less significance at the time the reflex disappeared than the blood pressure. Lack of oxygen and excess

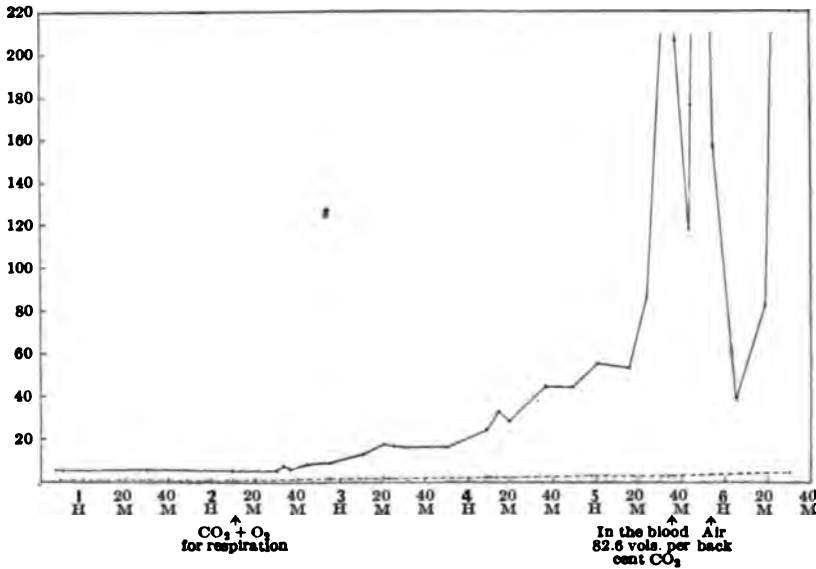


FIGURE 6. — Behavior of the reflex and the nerve-muscle preparation under excess of carbon dioxide and undiminished oxygen. Between 2.10 and 5.53, represented by the arrows, mixtures of the gases were administered by the Volhard method in gradually increasing amounts of carbon dioxide, beginning with 5 per cent carbon dioxide and 95 per cent oxygen, and ending with 30 per cent carbon dioxide and 70 per cent oxygen. Full line, reflex record; broken line, nerve muscle record. Ordinates, values of Z; abscissæ, elapsed time in hours and minutes.

of carbon dioxide might conceivably cause the circulatory system to become so inefficient that the blood would stagnate in the cord and, by thus lowering the amount of oxygen available to the cells, cause the reflex to disappear. Thus, not only with oxygen lack in the respired air, but also with excess of carbon dioxide, the loss of the reflex would really be due to lack of oxygen in the cord.

Numerous observers have reported that asphyxial conditions in animals with the central nervous system intact cause at first a rise in

¹⁰ HENDERSON, L. J.: this Journal, 1906, xv, p. 257.

blood pressure followed promptly by a fall. Mathison²⁰ has shown similar effects in spinal cats from lack of oxygen. With excess of carbon dioxide he found an initial fall followed by a rise.

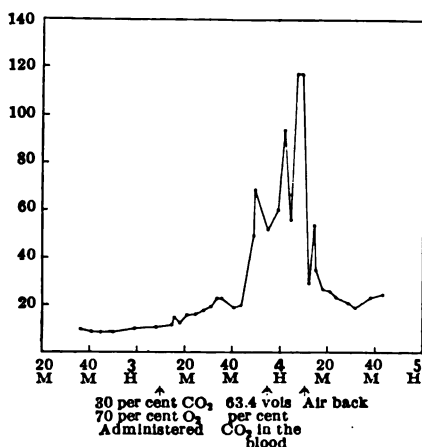


FIGURE 7.—Behavior of the reflex under excess of carbon dioxide and undiminished oxygen. Between 3.14 and 4.10, indicated by the arrows, a mixture of approximately 30 per cent carbon dioxide and 70 per cent oxygen was administered by the Volhard method. Ordinates, values of Z; abscissæ elapsed time in hours and minutes.

In the present research blood-pressure records have been secured for each of the different asphyxial conditions studied. The object has not been to make a thoroughgoing investigation of the relation between blood pressure and reflex irritability, but merely to show that the reflex might disappear without there being necessarily a fall in blood pressure, or, if the fall occurred, that it was at most relatively slight. First, however, in order to determine what should be considered a relatively slight fall, it was desirable to test the effect on the reflex of a lowered blood pressure without asphyxial complications, that is, with adequate

artificial respiration. Fig. 8 shows the results of such an experiment plotted in the form of curves. The low blood pressure was secured by bleeding from the carotid artery. The blood was defibrinated and later was returned to the circulation by injection into the jugular vein. The curve of blood-pressure changes is copied from the original record in order to facilitate easy comparison with the record of reflex irritability. In the blood-pressure record the ordinates represent millimetres of mercury. The records have been made from the carotid artery with a Hürthle manometer which was calibrated after each experiment.

Inspection of the curve shows that starting at 70 mm. the blood pressure sank to 25 mm. before the final decided rise in reflex threshold took place, and that with a minimum blood pressure of 21 mm. the threshold never went higher than 117 Z units.

²⁰ MATHISON: *Journal of physiology*, 1910, xli, p. 416.

Blood pressure and the reflex threshold under asphyxial conditions. — Fig. 9 shows a record of the behavior of the reflex under asphyxia, with a simultaneous blood-pressure record. It proves that the reflex may disappear in consequence of simple asphyxia while the blood pressure is still high. In this case the blood pressure was at 75 mm. of mercury when the experiment was begun and did not fall below 70 mm. until five minutes after the abrupt rise in reflex threshold. An essentially similar record has been secured of the blood pressure during a condition of oxygen lack without excess of carbon dioxide.

Fig. 10 is a record of an experiment in which the reflex was made to

disappear by excess of carbon dioxide. In this case, in contrast to that of diminished oxygen supply, there was no drop in blood pressure, but instead a very marked rise, which was maintained until several minutes after the reflex had disappeared, and showed no sign of falling, until the mixture of carbon dioxide and oxygen which was being administered was replaced by air. The blood pressure then dropped promptly to the normal level for the spinal cat. With an excess of carbon dioxide, therefore, low blood pressure is not essential for loss of the reflex.

It cannot be asserted, of course, that low blood pressure did not at times co-operate with an asphyxial condition of the blood to cause disappearance of the reflex. On the contrary, this undoubtedly occurred. Instances have been mentioned in which the heart ceased to beat at the same time that the reflex disappeared and just as the attempt was being made to draw a sample of blood for analysis. The reflex, in fact,

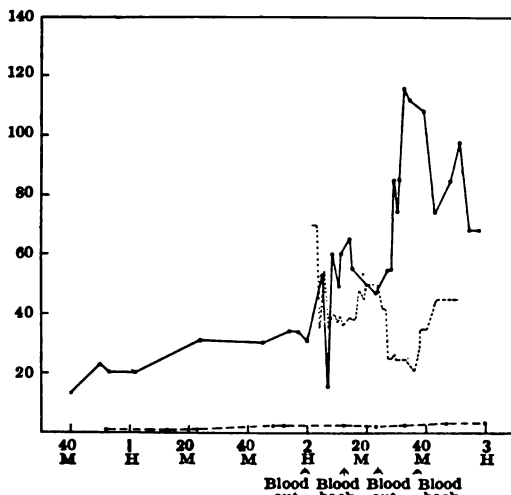


FIGURE 8. — Effect on the irritability of the reflex and the nerve-muscle preparation of a fall of blood pressure. Full line, reflex; broken line, nerve-muscle preparation; dotted line, blood pressure. Ordinates, values of Z and millimetres of mercury pressure; abscissæ, elapsed time in hours and minutes.

has sometimes been present for a moment or two after the heart beats could no longer be detected by palpation. In such cases the low blood pressure must have been an important co-operating factor in causing the disappearance of the reflex.

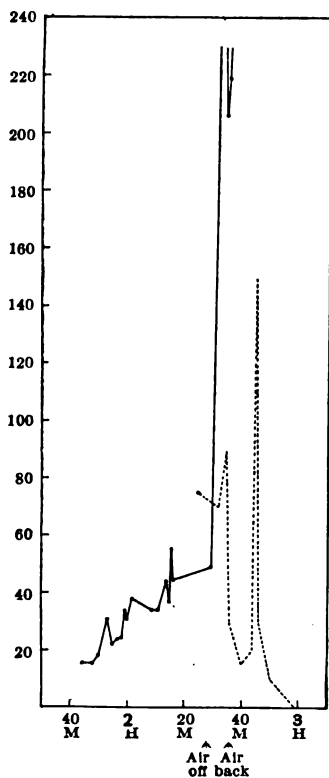


FIGURE 9. — Simultaneous record of blood pressure and reflex irritability during asphyxia. Notation as in Fig. 8.

Another fact in addition to the blood-pressure records which indicates that the reflex succumbs before actual stagnation of blood occurs is that the syringe into which the blood is drawn for the analysis is filled by the pressure of the blood itself which pushes up the plunger. If the attempt is made to draw blood into the syringe by pulling on the plunger, the carotid artery collapses and closes the opening into the cannula. Sufficient blood pressure to fill the syringe must be present, therefore, when the blood is drawn. The blood was always obtained just after the reflex had disappeared; hence there was always blood pressure at that time in those cases in which blood-gas analyses were successfully made. The conclusion seems justified that a low blood pressure was not a necessary condition for disappearance of the reflex, and that the blood-gas values secured were really the

significant factors in relation to that disappearance.

CONDITION OF THE NEUROMUSCULAR MECHANISM IN ASPHYXIA.

These asphyxial effects have all been described as changes in the threshold of the reflex. They might, however, conceivably be due to changes in the threshold of the neuromuscular mechanism which did not involve the cord. Inspection of Figs. 2, 4, 6, and 8 shows this not to be the case. In all these cases the irritability of the nerve-muscle

preparation is seen to have remained practically unchanged, although the reflex threshold either rose very considerably or was completely lost. In the case of simple asphyxia this is even more strikingly shown in an experiment which will best be made clear by quoting from the protocol.

Experiment of December 5, 1911.—Animal under artificial respiration as usual. Reflex threshold somewhat high, due to previous asphyxia. Threshold of the nerve-muscle preparation had not been above 4 Z units for an hour and a half. Data as follows:

Elapsed time.	Reflex threshold in Z units.	Nerve-muscle threshold in Z units.	Remarks.
h. m.			
3.05	10.5	Not determined	
3.09	7.9	Not determined	
3.15	11.6	Not determined	
3.18	Artificial respiration discontinued.
3.19	> 600.0	4.5	No reflex response from this point on.
3.25	> 600.0	2.4	
3.31	> 600.0	2.4	
3.36	> 600.0	2.4	
3.42	> 600.0	2.4	
3.45	> 600.0	2.6	Experiment discontinued.

In short, with the artificial respiration discontinued and after the reflex had disappeared, the nerve-muscle preparation retained its irritability practically without change for twenty-seven minutes.

THE QUESTION OF INCREASED IRRITABILITY UNDER ASPHYXIA.

Various investigators from time to time have noticed that reflexes were more easily elicitable in the first stages of asphyxia. Bethe²¹ states that a spinal dog when made to breathe hydrogen has an in-

²¹ BETHE: *Ergebnisse der Physiologie*, 1906, v, p. 283.

creased reflex irritability. Sherrington²² has observed that a certain degree of asphyxia favors the elicitation of the scratch reflex both in the decapitate and in the spinal animal, and Graham-Brown²³ is able

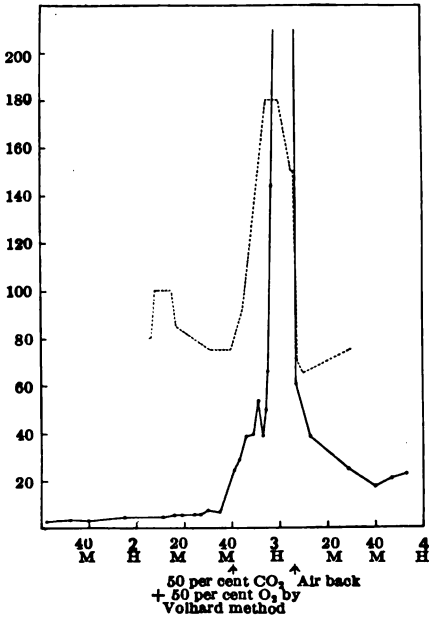


FIGURE 10. — Simultaneous record of blood pressure and reflex irritability during administration of carbon dioxide and oxygen. Notation as in Fig. 8.

to produce the scratch reflex in guinea-pigs by holding cotton over the nostrils and mouth for a few seconds when the reflex is otherwise difficult to elicit. Mathison²⁴ found that slight asphyxia favored the production of reflex movements. If the reflexes were not present, the breathing of nitrogen a few moments rendered them excitable. If present, asphyxia increased them enormously. Carbon dioxide, on the other hand, either had no effect or acted as a depressant.

The asphyxial increase of irritability would be expected to appear in curves secured in a study of asphyxia such as the present one, and would be indicated by a drop in the curve

just after asphyxia commenced, showing that a weaker stimulus sufficed to elicit the reflex. Examination of forty-seven records of the reflex under asphyxial conditions, whether brought about simply by cutting off the air supply or by diminishing the oxygen without allowing the carbon dioxide to accumulate, shows that the curve rose in thirty-six cases and fell in eleven cases, the fall as a rule being indicated by but a single reading, after which the curve either remained at the original level for several readings, then rose, or else rose immediately. See Figs. 2 and 4. A

²² SHERRINGTON: Quarterly journal of experimental physiology, 1910, iii, p. 213.

²³ GRAHAM-BROWN: Quarterly journal of experimental physiology, 1910, iii, p. 21.

²⁴ MATHISON: Journal of physiology, 1910, xli, p. 416.

similar examination of thirteen records of the administration of carbon dioxide shows that in ten cases the curve rose and in three cases only was there any indication of a drop. There is thus no decisive indication of heightened irritability under asphyxia in the data secured by the methods here employed.

A distinction should perhaps be drawn between "lowering of the threshold," that is, heightened irritability, and the "degree of the response." Thus, careful methods might indicate no lowering of a reflex threshold under certain conditions, and yet the threshold stimulus might under these conditions result in reflex contraction much more vigorous than normal. The conclusion drawn from increased discharge might be that the irritability was increased. In reality, however, the irritability has remained unchanged, but the *response* has increased. Of course this increase of response may involve lowered thresholds, that is, increased irritability, in the relations of the given reflex to neighboring neurons, but that need not alter the degree of the initial stimulus needed to call forth the slightest possible response. In a later paper it will be necessary to recur to this distinction.

The well-known asphyxial convulsions have uniformly appeared in the experiments with diminished oxygen supply. They are indicated in Figs. 2 and 4 by asterisks. Convulsions have not appeared with excess of carbon dioxide. This is in agreement with Mathison's observations.²⁵

SUMMARY.

1. A method is described by which the alterations in threshold of the flexion reflex and of a nerve-muscle preparation in the spinal cat are followed accurately while the animal is subjected to asphyxial conditions.
2. The blood-gas content of a spinal cat under artificial respiration averages: oxygen 11.5 vols. per cent, carbon dioxide 32.6 vols. per cent.
3. If the air supply is cut off from a spinal cat, there is little change in the threshold of the reflex for from two to four minutes. The threshold then rises abruptly and the reflex is lost. The blood-gas

²⁵ MATHISON: *Loc. cit.*, p. 439.

content at this time averages: oxygen 6.4 vols. per cent, carbon dioxide 30.6 vols. per cent.

4. If the oxygen supply be diminished, but the carbon dioxide be prevented from accumulating, the reflex is lost abruptly as before with a blood-gas content of oxygen averaging 4.5 vols. per cent and of carbon dioxide averaging 27.3 vols. per cent.

5. If oxygen be supplied in sufficient amount but carbon dioxide in excess, the results vary with the proportions used as follows:

(a) With 50 per cent carbon dioxide and 50 per cent oxygen the threshold rises very sharply and the reflex disappears, but with more delay than in the case of diminished oxygen. The blood-gas content when the reflex is lost is, in averages, oxygen 23.8 vols. per cent, carbon dioxide 47.2 vols. per cent.

(b) If a less proportion of carbon dioxide and correspondingly more oxygen are administered, the amount of carbon dioxide in the blood may reach 82.6 vols. per cent before the reflex is lost, or the reflex may not be lost at all, the carbon dioxide acting simply to raise the threshold to a greater or less degree.

6. The asphyxial effects are not always associated with any considerable fall of blood pressure, and in the case of excess of carbon dioxide the blood pressure may be abnormally high.

7. The threshold of the nerve-muscle preparation remains unaltered during even severe asphyxia.

8. The records secured offer no conclusive evidence of increased reflex irritability under asphyxial conditions.

THE INFLUENCE OF STANDING OR LYING UPON THE METABOLISM OF CATTLE.¹

By HENRY PRENTISS ARMSBY AND J. AUGUST FRIES.

[Investigations at the Institute of Animal Nutrition of the Pennsylvania State College in co-operation with the Bureau of Animal Industry of the United States Department of Agriculture.]

IN a number of previous publications² we have called attention to the very marked increase in the total metabolism of cattle in the standing as compared with the lying position which has been uniformly observed in our experiments. In thirty-seven published experiments this increase varied from a minimum of 28.3 per cent to a maximum of 64.5 per cent, averaging 41.4 per cent, and a considerable number of unpublished experiments have given entirely similar results. The foregoing figures refer primarily to that portion of the total heat which was given off by radiation and conduction and do not include that eliminated by means of the evaporation of water. On various grounds, however,³ it was assumed, in discussing the experimental results, that the proportion of the total heat produced which was carried off as latent heat of water vapor was essentially the same in the standing and lying positions, and the results of the experiments were computed upon the basis of this assumption.

Such an increase in metabolism as a result of standing is quite in accord with the results of numerous previous investigations, espe-

¹ Presented before Section 7 of the Eighth International Congress of Applied Chemistry. As presented before the Congress, the paper included only the results of Periods II, III, and V, but these were given in greater detail. In the present paper some minor corrections have been made in the figures for Period V, as well as a few verbal changes in the text.

² United States Department of Agriculture, Bureau of Animal Industry, Bulletins No. 51 (1903), p. 35; No. 74 (1905), p. 21; No. 101 (1908), p. 20; No. 128 (1911), p. 42.

³ Compare especially United States Department of Agriculture, Bureau of Animal Industry, Bulletin No. 128, pp. 122-124.

cially upon man. Thus Katzenstein ⁴ in experiments upon himself observed an increase in the oxygen consumption varying from a maximum of 32 per cent to practically zero when standing with the least possible muscular exertion. Zuntz ⁵ in experiments on a dog observed an increase of 40.9 per cent in the oxygen consumption and one of 36.5 per cent in the carbon dioxid excretion in the standing as compared with the lying position. Bornstein and Ott ⁶ in experiments on themselves found an increase of 6.6 cal. and 11.8 cal. per hour, respectively, in the computed heat production when standing over than when lying. Widlund,⁷ on the other hand, obtained but a slight increase in the carbon dioxid excretion in standing as compared with lying. Benedict and Carpenter,⁸ as the average of a number of experiments comparing sitting with standing, find an increase in the latter of 12 per cent for carbon dioxid, 16 per cent for oxygen, and 17 per cent for heat production. Hagemann ⁹ in two experiments on cattle reports increases of 23 per cent and 27 per cent in the total heat production in standing as compared with lying.

Quite recently, however, Zuntz ¹⁰ has called attention to the apparently small influence of standing and lying upon the metabolism of the sheep, observed in experiments by Hagemann ¹¹ in which the difference in the heat production as computed from the respiratory exchange was only 8 per cent. Dahm,¹² working in Zuntz's laboratory and by his methods, likewise found an increase of only 8 per cent in the respiratory excretion of carbon dioxid by a young bull when standing as compared with that when lying. Zuntz points out that even with a uniform rate of heat production the heat emission would tend to be less in the

⁴ KATZENSTEIN: Archiv für die gesammte Physiologie, 1891, xlix, pp. 361-362.

⁵ ZUNTZ: *Ibid.*, 1897, lxviii, pp. 194-195.

⁶ BORNSTEIN and OTT: *Ibid.*, 1905, cix, p. 621.

⁷ WIDLUND: Skandinavisches Archiv für Physiologie, 1905, xvii, p. 290.

⁸ BENEDICT and CARPENTER: Carnegie Institution of Washington, Publication 126, 1910, pp. 243, 253.

⁹ HAGEMANN: Landwirthschaftliches Jahrbücher, xli, Ergänzung, i, pp. 41, 128.

¹⁰ ZUNTZ: Medizinische Klinik, 1910.

¹¹ HAGEMANN: Archiv für Physiologie, Supplement Band, 1899, p. 111.

¹² DAHM: Biochemische Zeitschrift, xxviii, p. 494.

lying posture, since less of the animal's surface is exposed for radiation and evaporation, and is inclined to attribute the discrepancy between our results and those of Hagemann and of Dahm to this effect. In other words, Zuntz believes that the differences in the heat emission observed in our experiments are much exaggerated by a storing up of heat, during the intervals of lying, in the platform upon which the animal rests or in the body of the animal itself, this heat being given off again in the succeeding intervals of standing.

That there must be a tendency in this direction is undeniable, but we have not believed the effect to be of sufficient magnitude to affect seriously our comparisons of the metabolism in the two positions. There must necessarily be a limit to the amount of heat which can be stored up in the platform in this way, and it is easy to find in our experiments comparatively long intervals of lying in which, if Zuntz's explanation of the results be accepted, there must have been a storage in the platform of two or three times as much heat as it can be computed to have retained even upon the most extreme assumptions. Moreover, such an accumulation of heat in the platform would necessarily tend to increase radiation from the latter so that the heat emission during lying should tend to increase until it reached the level of the actual production by the animal. As a matter of fact, we have failed to find any distinct tendency of this sort in our experiments. Indeed, the heat emission during the periods of lying tends to be very uniform — more so, in fact, than during the periods of standing.

Obviously, however, arguments like the foregoing cannot be conclusive, and it still appeared possible that in comparisons between shorter intervals of standing and lying the difference in the heat emission as measured might be considerably greater than that indicated by a comparison of the gaseous exchange. In order to test this, appliances have been devised which permit the separate determination of the carbon dioxide and water vapor excreted in the intervals of standing and lying, respectively, and the present paper is a preliminary report upon the results obtained.

The experiments were made with the respiration calorimeter of the Institute, which is a modification of the original Atwater-Rosa apparatus for experiments on man.¹³ The apparatus is essentially a respi-

¹³ United States Department of Agriculture, Office of Experiment Stations, Bulletin No. 63 (1899).

ration apparatus of the Pettenkofer type with the addition of appliances for the determination of the heat emitted by the animal under experiment. A general description of the apparatus has been more than once given,¹⁴ and a recent publication¹⁵ describes in considerable detail the experimental methods pursued in these investigations. Repeated check tests in which pure alcohol was burned in the apparatus have shown that the results obtained may be regarded as accurate within the following percentages of the total amounts determined:

Carbon dioxid	0.5 per cent
Water vapor	6.0 " "
Heat	1.0 " "

For the special determinations of carbon dioxid and water vapor here reported, samples of both the ingoing and outcoming air are taken by means of rotary blowers. Each sample passes through an absorption train in which the carbon dioxid and water are absorbed and weighed, then through the blower, which is immersed in an oil bath, and finally through a calibrated metre (made by the Daansk Maalerfabrik, Copenhagen) which records the volume of the sample. By means of a series of valves coupled together the two currents of air may, at the instant when the animal stands up, be shunted from one absorption train and metre to another, while when he lies down again the current may be shunted back to the first series. In this way the aggregate excretion of carbon dioxid and water vapor for the experimental period in the two positions is determined. At each change of position samples of the air in the chamber of the apparatus are also taken for determination of the residual carbon dioxid and water vapor.

The results here reported were obtained in a series of experiments with a steer upon alfalfa hay and the so-called "alfalfa meal" (finely ground alfalfa hay). Each experiment extended over forty-eight consecutive hours (exclusive of five or six preliminary hours), covering the eighteenth and nineteenth days of a twenty-one-day feeding

¹⁴ United States Department of Agriculture, Bureau of Animal Industry, Bulletin No. 51 (1903); Experiment Station Record, No. 15 (1903-1904), p. 1037; Bureau of Animal Industry, Twenty-third Annual Report (1906).

¹⁵ United States Department of Agriculture, Bureau of Animal Industry, Bulletin No. 128 (1911), pp. 200-222.

period, and was divided into four subperiods of twelve hours each, the results for which are given separately. The rations consumed were:

Period I	7.5 kg. alfalfa hay
Period II	7.5 kg. alfalfa meal
Period III	6.0 kg. alfalfa hay
Period IV	6.0 kg. alfalfa meal
Period V	3.5 kg. alfalfa hay
Period VI	3.5 kg. alfalfa meal

The rations of Periods III and IV were estimated to be approximately maintenance rations. The subject was a pure-bred short-horn steer about twenty months old, in moderate condition, and weighing about 360 kg.

There were rather frequent changes of posture on the part of the animal, which would tend to emphasize any effect of a storing up of heat in the platform upon the partition of the heat emission between the standing and lying intervals.

In Period I there were twenty intervals of standing, varying from eight to one hundred and thirty-six minutes each, and twenty-one intervals of lying, varying from twenty to one hundred and thirty-three minutes each. In Period II there were seventeen intervals each of standing and lying, the length of the standing intervals varying from eighteen to one hundred and forty-seven minutes and that of the lying intervals from seventeen to one hundred and sixty minutes. In Period III there were twenty-one intervals of standing, varying from seven to one hundred and eleven minutes each, and twenty intervals of lying, varying from fifty-nine to one hundred and forty-six minutes each. In Period IV there were seventeen intervals of standing, varying from nine to one hundred and seventy-four minutes each, and sixteen intervals of lying, varying from two to one hundred and ninety-two minutes each. In Period V there were sixteen intervals of standing, varying from eleven to one hundred and eighty-six minutes each, and fifteen intervals of lying, ranging from seventy-three to one hundred and seventy-seven minutes each. In Period VI there were fifteen intervals each of standing and lying, the length of the standing intervals varying from seven to one hundred and sixty-six minutes and that of the lying from sixty-eight to one hundred and eighty-two minutes. Few of the intervals of either standing or lying, however, were less than ten minutes, the exception being eight in Period I, standing; seven in Period III, standing; nine in Period IV,

standing; two in Period IV, lying; and seven in Period VI, standing. The average length of the standing and lying intervals was:

	Standing	Lying
Period I	61 minutes	79 minutes
Period II	67 "	102 "
Period III	41 "	102 "
Period IV	64 "	111 "
Period V	61 "	126 "
Period VI	72 "	120 "

In addition to the separate determinations during the intervals of standing and lying, the total excretion of carbon dioxide and water vapor during each subperiod of twelve hours was also determined in duplicate in the customary way, namely, in a sample taken automatically by the metre pump (intermittent sampling) and in a continuous sample taken by a large aspirator having a capacity of about 800 litres. The results obtained on these two samples and the totals obtained by adding the results for the intervals of standing and lying showed a close agreement in nearly every instance, thus attesting the general accuracy of the work.

For each of the twenty-four twelve-hour subperiods, as well as for the whole of each period, the rate of elimination per minute of water vapor, carbon dioxide, total heat, and heat given off by radiation and conduction were computed from the totals found for the corresponding intervals. From these data the percentage increase observed in the standing over the lying periods was computed with the results shown in Table I.

It is clear that in general the increased emission of heat in the intervals of standing was accompanied by a correspondingly increased elimination of both carbon dioxide and water vapor, the parallelism being, on the whole, quite close, although with a tendency to a greater increase in the heat than in the carbon dioxide, which in a few instances, especially in Periods IV and VI, is somewhat marked. The same thing is shown in a different way by computing the heat emission per gram of carbon dioxide excreted as in Table II. We conclude, therefore, that the increased heat *emission* by cattle during standing, which has been invariably observed in our experiments, represents substantially the increase in heat *production* during the same time.

TABLE I.
PERCENTAGE INCREASE IN STANDING OVER LYING.

Period.	Position.	Subperiod 1.	Subperiod 2.	Subperiod 3.	Subperiod 4.	Whole period.
Period I	Carbon dioxid	32.9	25.4	26.2	26.8	27.4
	Water vapor	27.6	31.1	21.0	43.9	30.3
	Total heat	33.2	31.8	34.5	29.3	32.3
	Radiated heat	35.1	32.1	39.1	25.2	32.9
Period II	Carbon dioxid	32.8	30.7	21.6	28.6	28.2
	Water vapor	31.7	28.9	33.8	39.0	31.0
	Total heat	31.5	27.2	32.3	43.3	33.7
	Radiated heat	31.4	26.6	35.2	44.8	34.6
Period III	Carbon dioxid	35.1	33.1	39.8	34.6	35.1
	Water vapor	33.5	35.4	33.9	38.5	34.6
	Total heat	35.3	35.1	38.3	39.5	36.8
	Radiated heat	35.9	35.0	39.5	39.7	37.5
Period IV	Carbon dioxid	18.5	17.4	30.7	17.5	20.4
	Water vapor	30.2	28.0	28.7	31.0	28.4
	Total heat	38.8	34.6	30.9	34.1	34.0
	Radiated heat	41.4	36.6	31.6	35.0	35.7
Period V	Carbon dioxid	33.8	32.8	30.2	30.9	31.6
	Water vapor	25.4	48.9	29.6	42.3	34.6
	Total heat	33.5	44.2	38.8	39.5	38.4
	Radiated heat	35.8	43.0	41.1	38.8	39.4
Period VI	Carbon dioxid	36.5	12.4	28.5	20.5	24.0
	Water vapor	34.2	38.7	46.1	50.6	42.3
	Total heat	30.2	45.5	36.1	48.4	40.0
	Radiated heat	29.2	47.3	33.7	47.9	39.4

It will be noted that the influence of standing and lying upon the excretion of carbon dioxid was very much greater in these experiments than was observed by Hagemann or by Dahm. We are unable at present, however, to suggest any explanation of this difference, although it should be remembered that their results relate only to the

TABLE II.
TOTAL HEAT PER GRAM CARBON DIOXID.

Period.	Position.	Subperiod 1.	Subperiod 2.	Subperiod 3.	Subperiod 4.	Whole period.
Period I	Standing	2.400	2.399	2.453	2.218	2.373
	Lying	2.396	2.282	2.301	2.175	2.285
Period II	Standing	2.408	2.266	2.569	2.451	2.426
	Lying	2.431	2.330	2.361	2.200	2.327
Period III	Standing	2.390	2.295	2.451	2.451	2.402
	Lying	2.385	2.261	2.480	2.365	2.372
Period IV	Standing	2.595	2.603	2.402	2.669	2.572
	Lying	2.216	2.270	2.398	2.338	2.310
Period V	Standing	2.580	2.701	2.619	2.615	2.626
	Lying	2.584	2.488	2.455	2.454	2.498
Period VI	Standing	2.441	2.908	2.601	2.816	2.695
	Lying	2.550	2.247	2.455	2.286	2.387

respiratory excretion, while ours include also the cutaneous excretion and the carbon dioxid produced in the fermentations taking place in the alimentary canal.

That the calorific equivalent of carbon dioxid is in most cases materially lower than that corresponding to either protein, fats, or carbohydrates is doubtless to be ascribed to the very low thermal equivalent of the considerable amount of carbon dioxid produced in the methan fermentation, estimated by Zuntz* at 0.5 cal. per litre, equal to 0.255 cal. per gram.

* ZUNTZ: *Jahrbuch des Vereins der Spiritusfabrikanten in Deutschland*, 1912, xii, p. 328.

Finally, the computation, in Table III, of the percentage of the total heat which was given off as latent heat of water vapor shows

TABLE III.
PERCENTAGE OF HEAT GIVEN OFF IN WATER VAPOR.

Period	Position.	Subperiod-Subperiod 1.	2.	Subperiod 3.	Subperiod 4.	Whole period.
Period I	Standing	24.60	23.31	22.91	24.42	23.73
	Lying	25.66	23.44	25.46	21.95	24.10
Period II	Standing	24.50	23.33	23.83	25.56	24.31
	Lying	24.47	23.01	25.47	26.34	24.82
Period III	Standing	22.98	22.52	22.82	21.81	22.48
	Lying	23.29	22.48	33.36	21.96	22.86
Period IV	Standing	22.08	21.69	24.07	22.29	22.46
	Lying	23.53	22.80	24.46	22.80	23.44
Period V	Standing	20.26	20.96	18.74	20.01	19.88
	Lying	21.58	20.31	20.07	19.61	20.44
Period VI	Standing	21.40	20.49	20.89	20.72	20.85
	Lying	20.78	21.50	19.46	20.42	20.52

that this relation also was remarkably uniform, and fully justifies the assumption previously mentioned upon which we have based the computation of our earlier results.

ANTAGONISM BETWEEN SALTS AND ANÆSTHETICS. —
III. FURTHER OBSERVATIONS SHOWING PARALLEL
DECREASE IN THE STIMULATING, PERMEABILITY-
INCREASING, AND TOXIC ACTIONS OF SALT SOLU-
TIONS IN THE PRESENCE OF ANÆSTHETICS.

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I. INTRODUCTORY.

THE present paper forms a continuation of several previous contributions to the same general subject. In the experiments described in these papers the chief aim has been to obtain direct evidence of the part played by the semi-permeable limiting membranes of cells in physiological processes, especially in stimulation and in certain forms of toxic and antitoxic action. It has seemed probable that if changes in the permeability of the plasma membranes of the irritable elements form an essential part of the general process of stimulation,¹ as there is good reason to believe, any artificially produced modifications of irritability would be found associated with corresponding modifications in the properties of the membranes. Thus in narcosis or other conditions of hypo-irritability we should expect that the permeability of the membranes would undergo alteration with less readiness than normally, and that the cells would exhibit an increased resistance to those forms of injurious or toxic action which consist in the production of an abnormally increased surface permeability. Experiments on *Arenicola* larvæ and echinoderm eggs have shown, in fact, that the rapid permeability-increasing action of pure solutions of sodium chloride and other neutral salts is checked or prevented in the presence of anæsthetics, and that simultaneously

¹ Cf. this Journal, 1911, xxviii, p. 197; 1909, xxiv, p. 14.

the characteristic stimulating and toxic effects of these solutions undergo a marked decrease.²

Narcosis, as a physiological phenomenon, is interesting chiefly because of the uniformity both of its manifestations and of the conditions under which it is produced. Activities of the most various kinds (cell division, stimulation and conduction, contractile processes, secretion, growth) are diminished or abolished, reversibly, in the presence of certain substances. Of these the most universal in their action are the numerous largely water-insoluble organic compounds possessing solvent action on fats and lipoids. Since protoplasm appears always to contain lipoids, a selective action on these substances seems thus to be indicated as the basis of anæsthesia. Overton and Meyer found that a parallelism between the water-lipoid partition coefficients of the lipoid-solvent anæsthetics and their narcotic action did in fact exist, and they accordingly referred narcosis to an alteration of the lipoids. The question as to why lipoid alteration should decrease or abolish irritability remained, however, unsettled. Narcotic action is also exerted by various substances — salts, non-electrolytes, acids — which have no specific relation to lipoids, and also by the electric current under certain conditions (anelectrotonus). Apparently, therefore, specific alteration of the lipoids is not the essential basis of narcosis, although such a change may induce this state. The indications point toward some more general kind of modification in the irritable elements; this modification may be caused by, but is not necessarily connected with, a change in the condition of the lipoids.

In anæsthesia we have to do with a change in irritability. The problem of the physiological nature of anæsthesia is thus inseparable from the more general problem of the nature of the stimulation process. The influence of anæsthetics on irritability must therefore be studied from the general point of view of this latter problem. It should be noted that these substances do not invariably *lower* irritability, but do so only in certain strengths of solution; below these concentrations they frequently increase the responsiveness or degree of automatic activity of the tissue. Thus traces of ether and other anæsthetics increase the rate of the heart beat, of growth and cell

² Cf. this Journal, 1912, xxix, p. 372; xxx, p. 1.

division, of ciliary movement, of amoeboid movement.³ In higher concentrations these activities are checked or brought to a rest, reversibly. In still higher concentrations cytolytic action sets in. There are thus ranges of concentration corresponding to quite different physiological effects. The same phenomenon is seen with certain salts — for example, those of calcium. Irritability may thus be modified in either direction; an irritable tissue may be sensitized or desensitized by appropriate concentrations of a given lipid solvent or other substance. The terms “sensitization” and “desensitization” may have reference not only to changes in the responsiveness of tissues or organisms to stimulation, but also to changes of susceptibility to particular poisons. This is probably no merely verbal coincidence. A parallelism between the two effects, irritability-increasing and susceptibility-increasing, exists in certain cases,⁴ and there is evidence that the essential physico-chemical basis of both kinds of effects is the same, and consists in an alteration in the normal properties of the plasma membranes, of such a kind that the increase of permeability essential to both the stimulating and the toxic action of the poisonous substance (*e. g.*, saponin) is facilitated or promoted. Conversely, anæsthetics desensitize the tissues of *Arenicola* larvæ to both the stimulating and the toxic action of pure isotonic NaCl solutions.

There now exists ample evidence that the initial or critical event in stimulation consists in a change in the condition of the surface film or plasma membrane of the irritable element. The passage of an electrical current through the tissue necessarily causes a change in the electrical polarization of the semi-permeable membranes, and it is this change (as shown by Nernst and his successors) which forms the critical or primary process upon which follow the chemical, mechanical, or electrical manifestations (or response) characteristic of the tissue. It is well known, however, that mechanical impact, heat, and various chemical substances may call forth in a nerve or muscle

³ Instances of these effects are cited in the first paper of this series this: *Journal*, 1912, *xxix*, p. 374.

⁴ *E. g.*, the toxic action as well as the stimulating action of saponin and other poisons on frogs' voluntary muscle is greatly increased if the muscle is previously “sensitized” by immersion for a few minutes in a pure isotonic solution of a sodium salt. *Cf.* this *Journal*, 1911, *xxviii*, p. 214.

the same response as the electrical current; the essential or determinative change in stimulation must therefore be one which is produced in common by all of these agencies. Now the facts of electrical stimulation, significant as they are, do little more than *localize* this primary or critical action at the semi-permeable membranes of the tissue; the precise nature of the change there produced has to be determined by other means; and it remains to inquire what kind of surface change is adequate to account for the most characteristic and constant features of stimulation, as well as for the possibility of its being caused by so many different agencies. In brief, the evidence indicates that the properties of the limiting membranes undergo temporary alteration during stimulation; the plasma membranes, semi-permeable during rest, appear during stimulation to become more permeable than before.⁵ Stimulation, in other words, is associated with an increase in the general permeability of the limiting membranes. This consideration explains why agencies other than the electric current stimulate; they act by directly altering the permeability of the membrane. Such a change would account for the electrical variation which so constantly accompanies stimulation, and also for such characteristic effects as the refractory period, as well as for the osmotic effects which appear in some cells (gland cells, pigment cells, egg cells after fertilization) and indeed in certain cases, as in motile plant tissues, form the essential condition of the response.

If this hypothesis is well based, the condition of the membrane at any time must affect the readiness with which its permeability undergoes change, and hence also the responsiveness of the tissue to stimulation. This consideration explains the characteristic effectiveness of lipid solvents in altering irritability. The general properties of plasma membranes indicate that lipoids enter largely into their composition. Both animal and plant cells are as a rule readily permeable to lipid solvents and lipid-soluble substances, as Overton was the first to show; and these substances in higher concentrations have a specific action in increasing the permeability of the membranes. If the characteristic properties of the plasma membranes depend largely on the condition of the component lipoids, it is to be expected that lipid-modifying substances will influence the readiness with which

⁵ For a general review and theoretical discussion of this evidence cf. my paper in this Journal, 1911, *loc. cit.*, p. 197.

the permeability of the membrane is altered by various agencies, and also — if an increase of permeability is essential to stimulation — that they will at the same time modify the readiness with which stimulation is effected. These two effects ought theoretically to show a certain parallelism. In other words, any anæsthetic which decreases the susceptibility of a tissue to stimulation ought to decrease at the same time its general susceptibility to permeability-increasing or cytolytic action. The experiments about to be described show that this is in fact the case, so far as regards the action of neutral salt solutions on the tissues of *Arenicola* larvæ.

In an investigation like the present, which aims at determining the relation between permeability alteration as influenced by anæsthetics and stimulation as influenced by the same substances, it is indispensable to obtain as object of experiment an organism which will show simultaneously and in a clear and unmistakable manner both the changes of permeability and the stimulating effects caused under given conditions, *e. g.*, by a certain solution. Higher organisms are obviously unsuitable for this purpose; in these the differentiation is too detailed and the physiological conditions are too complex. Such a satisfactory test organism — or physiological indicator — is to be sought only among the lower forms of life, and I have found the free-swimming larvæ of the common shore annelid *Arenicola* remarkably well adapted to this purpose. These organisms have the advantages of being readily obtainable in large quantities, of showing great constancy in their reactions, and of possessing a sufficient but not too detailed differentiation of cells and tissues. The musculature is well developed and responds normally to electrical and other forms of stimulation — *e. g.*, the law of polar stimulation is shown typically; external locomotor cilia are present, and a yellow pigment (appearing brown when the larvæ are massed) is abundant in the body cells, and passes readily into the water when the permeability is increased by cytolytic or other agencies; this substance thus serves as a convenient index of the degree of permeability increase.

I have elsewhere described the action of pure isotonic NaCl solution on *Arenicola* larvæ.⁶ The visible effects which immediately follow the addition of the solution and indicate most clearly the essential nature of its primary action may be described as of three chief kinds,

⁶ Cf. this Journal, 1909, xxiv, p. 23.

relating respectively to the musculature, the pigment-containing cells, and the cilia. These effects are briefly as follows: (1) There is a strong and persistent muscular contraction, causing the larvæ to shorten to about half their normal length; they remain thus contracted for twenty or thirty seconds and relax slowly, regaining their original length within about a minute after the first contact of the solution; all muscular movements then cease, and during their further stay in the solution the larvæ remain quite motionless. (2) The yellow pigment contained in the body cells diffuses rapidly into the solution, and if sufficient larvæ are present imparts to this a distinct straw-yellow tinge. (3) The locomotor cilia cease movement almost immediately, and shortly afterwards many undergo a visible disintegration or breakdown into minute droplets or granules; usually there is some variation in resistance, and a few cilia may continue feeble movement for a minute or two and occasionally longer; but typically all trace of activity ceases within five minutes or less, and there is no revival when the larvæ are returned to sea water. A stimulating, a permeability-increasing, and a direct disintegrative action can thus be distinguished. In addition to these visible external effects there is a general toxic action affecting the entire organism. Larvæ returned to sea water after a few minutes (less than five) in the pure NaCl solution are found to have permanently lost their normal heliotropism; muscular contractions return after an interval, but slowly and imperfectly; there is no revival of ciliary movement, and the larvæ die within a few hours. If the return to sea water is delayed for several hours, there may be no signs of revival.

The above effects indicate that the salt solution acts primarily on the solid colloidal structures, especially the surface structures, of the tissues. Apparently the colloids undergo immediate changes of aggregation state, which lead in the case of the cilia to a structural breakdown and in the case of the tissue cells to an abnormal increase in the permeability of the limiting membranes. The latter lose — at least temporarily — their insulating or semi-permeable properties; hence a certain loss of material results, as indicated by the loss of pigment; at the same time the muscle cells undergo intense stimulation, and a well-marked toxic or generally disorganizing action sets in. These last two effects appear to be direct consequences of the change in permeability; this is indicated by the close parallelism which

exists between the degree of the permeability-increasing action of salt solutions and both their stimulating and toxic action. Certain substances decrease or prevent the permeability-increasing action of the pure salt solution; among those are not only various neutral salts — *e. g.*, those of calcium and magnesium — but also, as I have recently found, a large number of lipoid-solvent anæsthetics; at the same time they decrease or prevent the immediate stimulating action of the solution and lessen its toxic action. This parallelism indicates that the semi-permeable surface films or plasma membranes of the cells form the *locus* both of the stimulating and of the toxic action of the salt solution, and that the above substances exert their anti-stimulating and antitoxic action by altering the plasma membranes in such a way as to decrease or prevent the permeability-increasing action on which both effects depend.

Direct observation thus indicates that modification of surface structures forms the basis of certain characteristic physiological effects produced by neutral salts and anæsthetics in these organisms. The pure salt solution causes visible changes in the physical state of the colloidal filaments and membranes at the cell surfaces. These effects, in so far as they are irreversible, are toxic. Thus it is immediately apparent why pure sodium chloride solution arrests ciliary movement; the colloids of the tissue are so altered as to *interrupt the continuity* of the contractile filaments; the latter then necessarily cease movement and undergo breakdown. In such a case the effect is essentially irreversible. Presumably similar changes take place in the plasma membranes of the cells; obviously any such interruption in the continuity of the membranes would necessarily involve marked increase of permeability. The case of the cell, however, differs from that of colloidal filaments like cilia in that there always remains the possibility that the continuity of the surface film may be restored by deposition of materials from the protoplasm. Possibly this is why the destructive action of the pure salt solution on the cells (*e. g.*, muscle cells) of this organism is more gradual than on the cilia, so that some recovery is possible after relatively prolonged exposure. It seems clear that the preservation of the continuity and semi-permeability of the surface films is essential to the continuance of normal vital processes; hence the presence of substances that favor this condition protects the cell against injury by toxic substances or other destructive agencies.

II. EXPERIMENTAL.

In the experiments described in the present and preceding papers I have tested the action of a variety of anæsthetics in modifying the above-described stimulating, permeability-increasing, and toxic action of the pure isotonic NaCl solution. The substances used include the following: a series of alcohols, methyl, ethyl, n-propyl, iso-propyl, n-butyl, n-amyl, n-capryl; chloretone; a series of esters, — ethyl acetate, propionate, butyrate, valerianate, nitrate; methyl, ethyl, and phenyl urethanes; various normal and substituted hydrocarbons, — carbon tetrachloride, chloroform, nitromethane, acetonitrile, benzol, toluol, xylol, phenanthrene, naphthalene; and a number of miscellaneous compounds, — ethyl ether, chloral hydrate, chloralose, paraldehyde, phenyl urea, acetanilide, phenacetin, methacetin. The action of certain inorganic salts (as of magnesium and calcium) and of isotonic sugar solution falls partly into the anæsthetic category, but will not be considered in the present paper.

In these experiments the solution, 0.55 m. NaCl, containing the anæsthetic in known concentration, is added suddenly to a mass of larvæ which have been collected in a watch glass or in the interior of a small flask. The immediate effects of the solution on muscular and ciliary movement and the degree of revival in sea water after known periods of exposure, can be readily determined and compared with the corresponding effects of the control solution (pure 0.55 m. NaCl).

In general it has been found that all anæsthetics — provided their solubility in salt solution is sufficient — show the following effects in common. In concentration above certain maxima they cause rapid cytolysis, and reinforce the disintegrative action of the pure salt solution. In weaker solutions, within a certain range of concentrations characteristic for each anæsthetic, they exhibit a definite anti-stimulating and anti-cytolytic action. The degree of this action varies with different anæsthetics, but is constant and characteristic for any particular compound. Usually the muscular contraction on the first contact with the anæsthetic-containing solution is much less intense and prolonged than in the pure salt solution, and in many cases it is completely or almost completely prevented. The immediate loss of pigment is also usually decreased, and in many cases en-

tirely prevented; the degree of this effect shows a close parallelism with that of the decrease of stimulation. Ciliary movement is almost always decidedly prolonged — in favorable cases for several hours. If after a certain stay in the solution the larvæ are returned to sea water, they are found to recover muscular contractility more rapidly and completely than after a similar exposure to pure 0.55 m. NaCl. The general disintegration produced by the salt is also lessened or retarded in the presence of the anæsthetic, and the larvæ remain alive longer after return to sea water. The anæsthetic thus shows a definite protective or antitoxic action similar to that of calcium chloride and other salts; this effect is highly characteristic and has been shown without exception by every substance so far tried which exhibits definite anæsthetic action in sea water.

The concentrations most favorable for the prevention of the stimulating and cytolytic effects of the salt solution correspond closely with those which produce typical anæsthesia in normal larvæ in sea water. Hypo-anæsthetic concentrations always fail to prevent, though they may decrease, the immediate stimulation and loss of pigment; but in most cases, unless too low, they exert a distinct protective or antitoxic action, — *i. e.*, they retard the disintegrative or cytolytic action of the salt solution, and on return to sea water from such solutions recovery is found to be more complete than after similar exposure to the pure salt solution. Another remarkable and characteristic effect of anæsthetics in hypo-anæsthetic concentrations is to *prolong* the period during which muscular contractions continue in the salt solution; in pure 0.55 m. NaCl all contractions typically cease within four minutes or less, and the larvæ remain quite motionless during their further stay in the solution; but in the presence of the anæsthetic slight contractions often continue steadily — in many cases for half an hour or more. This effect recalls the stimulating or sensitizing action so characteristic of anæsthetics in weak solution; but it seems rather to be the expression of a certain protective or antitoxic action which enables the tissue partly to preserve its normal properties even in the otherwise strongly toxic pure salt solution. The presence of traces of calcium chloride has a similar effect.

ACTION OF SPECIAL ANÆSTHETICS.

Alcohols. — All of the above-named alcohols in favorable concentrations decrease or prevent the initial stimulating and permeability-increasing action of isotonic sodium chloride solution, protect the cilia against immediate breakdown, thus prolonging their activity, and retard the general toxic action. The optimum concentrations for the immediate anti-stimulating and anti-cytolytic actions are in general the same; as already said, they correspond closely with the concentrations which produce typical anæsthesia in sea water. These concentrations show the usual progressive decrease with increase in the molecular weight of the alcohol. In physiologically equivalent solutions the different alcohols modify the action of the salt solution in essentially the same manner. If the proportion of alcohol is favorable the larvæ show no immediate loss of pigment and little or no stimulation; either no evident muscular contraction occurs on the first contact with the solution — the larvæ merely ceasing movement and remaining relaxed and motionless — or the resulting contraction is slight or moderate and followed by immediate relaxation and complete cessation of movement. In such solutions the cilia typically continue their movement. In concentrations of alcohol somewhat higher than optimum the cytolytic action of the solution is eventually accelerated, though there may be typical protective action at first. In still stronger solutions the cilia cease at once, and cytolysis is rapid from the first. There are certain specific differences of toxicity between the different alcohols; propyl and butyl alcohols have shown the most favorable, *i. e.* most completely reversible, protective action, while capryl alcohol always shows a marked specific toxicity, even in concentrations that at first appear highly favorable. Chloretone, which may be classed with the alcohols, is remarkably rapid and complete in both its anti-stimulating and anti-cytolytic action, as already described.⁷

The following is a summary of the observations made with alcohols. The control solution with which comparison is made is always the pure 0.55 m. NaCl.

⁷ This Journal, 1912, xxix, p. 387.

1. **Methyl alcohol.** — A solution of 10 vol. per cent in 0.55 m. NaCl causes a well-marked immediate contraction (though less than the control) followed by instant relaxation; no further muscular contractions are seen; the cilia remain active for half an hour or more. A little pigment is liberated at first, but much less than in the control.

Effect of return to sea water. — After forty-five minutes in the solution the larvæ recover muscular contractions much more completely than in the control; the cilia also revive in part; after three hours' exposure recovery is also greater than in the control, but the difference is less; after six hours there is no recovery either in the solution or in the control.

In 5 vol. per cent solution strong stimulation and well-marked exit of pigment occur essentially as in the control. The cilia are protected only slightly, and most cease movement within five minutes. Slow muscular contractions continue for an hour or more in this solution.

Effect of return to sea water. — The protection is at first less marked than in the 10 per cent solution, but lasts longer. After intervals of three and six hours active contractions return in sea water; even after twenty-two hours in the solution many larvæ recover slightly.

Anæsthetic concentration in sea water. — This is *ca.* 10 vol. per cent. There is no muscular movement in this solution and recovery is well marked after eighteen hours. In 5 vol. per cent solution contractions continue actively.

2. **Ethyl alcohol.** — *a.* 10 vol. per cent. There is a slight muscular contraction at first and no immediate loss of pigment. The cilia cease at once. After two and one-half hours cytolysis is well marked.

Return to sea water. — In larvæ transferred after twenty-three minutes and forty-five minutes the cilia revive and well-marked contractions return; after two and one-half hours there is no revival.

b. 5 vol. per cent. There is moderate contraction at first with instant relaxation. No loss of pigment is evident. Cilia continue for some time. The muscular anæsthesia is not quite complete; occasional slight contractions persist for some time.

Return to sea water. — Larvæ returned after forty-five minutes recover almost completely; some show heliotropic swimming, though the direction of the response is reversed (negative). After two and one-quarter and five hours active contractions return, much greater than the control. A fair proportion recover contractility after twenty-one and one-half hours in the solution.

Anæsthesia in sea water. — In sea water with 6 vol. per cent alcohol

muscular anæsthesia is practically complete. The effect is perfectly reversible. In 5 vol. per cent. slight contractions continue; 10 vol. per cent is rapidly toxic.

3. *n*-propyl alcohol. — Solutions in 0.55 m. NaCl of the following concentrations were used: 5, 4, 2.5, 2, 1.25, 1, 0.63, and 0.5 vol. per cent. In general the effects were similar to those seen with ethyl alcohol, but somewhat more favorable. In the stronger solutions down to 2 vol. per cent (inclusive) there is almost no immediate stimulation or loss of pigment. In 1.25 and 1 vol. per cent stimulation is stronger and there is slight immediate loss of pigment (< control); in 0.63 and 0.5 vol. per cent stimulation and exit of pigment are well marked. The protective action on cilia is decided down to 2 vol. per cent (in 2.5 vol. per cent solution some ciliary movements remained after seventeen hours); below this it is less distinct or absent. In 1.25 per cent and weaker solutions muscular anæsthesia is incomplete and contractions continue for some time — in some cases for more than an hour.

Return to sea water. — In the 5 vol. per cent solution the larvæ, though protected at first, die within an hour; in the 4 per cent solution cytolytic changes appear within one and a half hours. Revival after one hour in this solution is greater than in the control. Larvæ from the 2.5 per cent solution show a remarkably prompt and perfect recovery after an exposure of forty minutes, and many recover helio-tropism (mostly negative); the same was observed with the 2 per cent solution after one hour's exposure, and with the 1.25 per cent after thirty minutes. In the weaker solutions protection is at first less complete — *i. e.*, the initial injury is greater — though it remains evident after longer periods of exposure. After seventeen hours' exposure larvæ from 2.5, 1.25, and 0.63 vol. per cent showed good revival of contractions; the same was true of 1 and 0.5 per cent solutions after twenty-two hours' exposure.

Anæsthetic action in sea water. — The minimum concentration is between 2 and 3 vol. per cent; arrest of contractions is not quite complete in 2 vol. per cent solution.

4. Isopropyl alcohol. — This was used in the concentrations 4, 3, 2, and 1 vol. per cent, and showed essentially the same action as the normal alcohol, although in equivalent concentrations both its anæsthetic and toxic actions appeared somewhat weaker. In 4 and 3 vol. per cent solutions immediate stimulation is slight and there is no loss of pigment; in 2 vol. per cent stimulation and loss of pigment are well marked, though less than in the control; in 1 vol. per cent there is little difference from the control. Protective action both on muscle and cilia is

well marked, — best in 3 vol. per cent and relatively slight in 1 vol. per cent.

5. *n*-butyl alcohol. — The following solutions were used: 4, 2.5, 2, 1.25, 1, 0.62, and 0.31 vol. per cent. The same effects were observed as with propyl alcohol, but the physiologically equivalent concentrations were in all cases lower: 4 vol. per cent causes rapid cytolysis and breakdown of cilia; 2.5 vol. per cent causes rapid cytolysis within fifteen minutes, but prevents the immediate stimulation and loss of pigment; 2 vol. per cent prevents stimulation and loss of pigment and preserves ciliary movement for an hour or more, but is fatal within two hours. In 1.25 and 1 vol. per cent solutions the protective and anti-stimulating actions are best marked; there is little or no immediate stimulation and no exit of pigment, and ciliary movement lasts for an hour or more; 0.62 vol. per cent acts similarly; a few cilia remain active after eighteen hours in this solution, but muscular anæsthesia is not quite complete; in 0.31 vol. per cent stimulation and pigment exit are well marked, though less than the control; the cilia cease in a few minutes, while muscular contractions continue.

Return to sea water. — Larvæ returned to sea water after fifty-five minutes in 2.5 vol. per cent alcohol showed no revival. After a similar stay in 1.25, 1, and 0.62 vol. per cent recovery of contractions was prompt and complete, and many larvæ showed a return of the normal positive phototaxis (especially with 1.25 and 1 vol. per cent); after 0.31 vol. per cent recovery of contractions was slower and less complete and the cilia failed to revive. Muscular contractions reappeared after eighteen hours in the 1 and 0.62 and 0.31 per cent solutions (best with 0.62 per cent), but not in 1.5 per cent (no revival in control).

Anæsthetic concentration in sea water. — The minimum is *ca.* 0.8 vol. per cent, which causes complete muscular anæsthesia without arresting the cilia.

6. *n*-amyl alcohol. — The following solutions were used: 1, 0.5, 0.4, 0.25, 0.2, 0.13, and 0.1 vol. per cent. In 1 vol. per cent solution cytolysis is rapid. In 0.5, 0.4, 0.25, and 0.2 vol. per cent the initial stimulation is slight and there is no loss of pigment; in 0.13 per cent the initial stimulation and pigment exit are well marked, though less than in the control; 0.1 vol. per cent shows little difference from the control. In 0.5, 0.4, 0.25, and 0.2 vol. per cent breakdown of cilia is prevented and ciliary movement is prolonged for about an hour; in 0.13 and 0.1 per cent the cilia cease almost as soon as in the control. Muscular anæsthesia is incomplete below 0.25 vol. per cent, and slight contrac-

tions continue in these solutions (0.2, 0.13, 0.1 per cent) for some time.

Return to sea water. — One vol. per cent is quickly destructive. In the case of the other solutions larvæ returned within one hour to sea water recovered much more completely than the control, but phototaxis was not renewed; 0.1 vol. per cent is the least favorable. With prolonged exposure (eighteen hours or more) larvæ from 0.5 vol. per cent showed no revival, those from 0.4 vol. per cent revived only slightly (again the stronger solutions, though showing better protection at first, are less favorable than the weaker when the exposure is prolonged); larvæ from 0.25, 0.2, and 0.13 vol. per cent showed well-marked renewal of contractions, and from 0.1 per cent slight renewal. There was no recovery in the control.

Anæsthetic concentration in sea water. — The minimum for complete muscular anæsthesia is *ca.* 0.25 vol. per cent.

7. *n*-capryl alcohol. — Capryl alcohol ($C_8H_{17}OH$) is only slightly soluble in water (1 part in *ca.* 2000). The solutions used (in 0.55 m. NaCl) were: saturated (*ca.* 0.05 per cent), three-fourths saturated, half saturated, and three-eighths, one-fourth, and one-eighth saturated. In the saturated solution the immediate contraction and loss of pigment are completely prevented, but within fifteen minutes cytolysis sets in; in half and three-fourths saturated there is also little or no immediate stimulation and no exit of pigment, and the cilia continue their activity (in some larvæ for almost three hours); in the three-eighths saturated the initial contraction is well marked, there is slight loss of pigment, and the cilia cease soon; in the one-fourth and one-eighth saturated solutions the initial contraction and pigment exit are pronounced, and protection to cilia is slight or absent; in these two solutions muscular contractions continue for some time.

Return to sea water. — Protective or antitoxic action is well marked at first in all solutions except the saturated. After three hours in the half, three-eighths, and one-fourth saturated solutions the revival of contractions in sea water was decidedly greater than in the control; one-eighth saturated showed relatively little action. After six hours' exposure there was slight revival in larvæ from the half and three-eighths saturated solutions, but not from the others. There was no revival after twenty-two hours. The strong specific toxicity of this alcohol asserts itself with the longer exposures, although typical antitoxic effects are plainly evident at first.

Anæsthetic concentration in sea water. — The optimum anæsthetic concentration is *ca.* one-third saturated; half-saturated solutions are

fatal within an hour, while with one-fourth saturation muscular anæsthesia is not quite complete. This alcohol has a high specific toxicity; after eighteen hours all of the larvæ in the one-third saturated and most in the one-fourth saturated were found dead.

Fatty acid esters. — To save space a less detailed account of the observations with these compounds will be given. Their general action is similar to that of the alcohols, but the toxicity is greater (due probably to hydrolysis) and the protective action less. The four ethyl esters, acetate, propionate, butyrate, and valerianate, were used. As with the alcohols, the favorable anæsthetic and protective concentrations decrease progressively with increase in the molecular weight. In one respect these esters — especially the higher members — differ from most of the anæsthetics investigated in these experiments in showing a well-marked specific or selective action on the pigment-containing cells of this organism. Solutions of ethyl butyrate and valerianate in sea water, in favorable anæsthetic concentrations, extract considerable pigment from the larvæ; ethyl propionate shows this action to a less degree, while the acetate anæsthetizes without loss of pigment. In correspondence with this peculiarity the two higher esters do not prevent the immediate loss of pigment in 0.55 m. NaCl, although they inhibit stimulation and retard toxic action in the same manner as the others.

Action of esters in 0.55 m. NaCl. — 2.5 vol. per cent solutions of ethyl acetate are rapidly fatal. In 2, 1.5, and 1 vol. per cent solutions there is only slight immediate stimulation and little or no loss of pigment, and ciliary movement lasts decidedly longer than in the pure salt solution. In 0.5 and 0.25 vol. per cent solutions there are strong contraction, marked loss of pigment, and immediate cessation of ciliary movement; slow muscular contractions continue in these solutions for some time.

Transfer to sea water. — Well-marked protective action is shown by all of the above solutions from 2 vol. per cent down; but in the stronger solutions, 2 and 1.5 vol. per cent, it lasts for only a short time; after a few hours in these solutions there was no recovery. After four hours in the 1, 0.5, and 0.25 per cent solutions revival of contractions was much more complete than in the control (0.5 vol. per cent proved the most favorable).

Anæsthetic concentration in sea water. — Solutions of 1 to 1.5 vol. per cent produce complete typical muscular anæsthesia in sea water.

Similar effects are seen with the other three esters, the physiologically equivalent concentrations decreasing with increase in the molecular weight. The anæsthetic concentrations in sea water are approximately as follows: propionate, 0.4 to 0.5 vol. per cent; butyrate, 0.13 to 0.25 vol. per cent; valerianate, 0.13 to 0.08 vol. per cent. Solutions of these concentrations in 0.55 m. NaCl show typical anti-stimulating and anti-cytolytic action, and prevent the immediate breakdown of cilia; stronger solutions cause cytolysis, while in weaker solutions stimulation and ciliary breakdown occur essentially as in the pure solution, and muscular contractions are prolonged. Butyrate and valerianate are relatively inefficient in preventing the immediate exit of pigment, though showing typical anti-stimulating and antitoxic action.

Other esters examined include ethyl nitrate and the urethanes (methyl, ethyl, and phenyl). The following is a brief account of the observations with these compounds.

Ethyl nitrate. — Solutions (in 0.55 m. NaCl) of the concentrations 0.75, 0.5, 0.35, and 0.25 vol. per cent were used. This ester shows strongly marked anti-stimulating and anti-cytolytic action. In all of the above concentrations the immediate stimulation and loss of pigment are slight or absent, and ciliary movement continues; in 0.75 vol. per cent a gradual cytolysis begins within a few minutes, and the larvæ show little or no recovery in sea water after an hour's exposure; in the 0.5 per cent solution the anæsthetic and protective actions are typical and lasting; in 0.35 and 0.25 per cent muscular anæsthesia is only partial; and slow contractions continue for some time.

Return to sea water. — After half an hour in the above solutions all of the larvæ recovered much more completely than in the control, and a certain proportion of those from 0.5 vol. per cent showed negative phototaxis; of the larvæ transferred to sea water after five hours in the last three solutions, all showed active contractions next day, while all from the control and from 0.5 vol. per cent were dead.

Anæsthetic concentration in sea water. — One vol. per cent causes rapid cytolysis; 0.35 and 0.5 vol. per cent produce typical reversible anæsthesia; in 0.25 vol. per cent muscular anæsthesia is incomplete, and slight contractions continue.

Ethyl urethane. — In my former paper experiments with this ester were described, but the strongest solution used, 0.6 per cent, was insufficient to prevent stimulation and loss of pigment in 0.55 m. NaCl. In higher

concentrations urethane shows this action typically. In 4 per cent and 2 per cent solutions immediate loss of pigment is prevented, stimulation is greatly diminished and the cilia continue activity — in the 2 per cent solution for an hour or more. In 1 per cent urethane there are considerable immediate stimulation and loss of pigment, — though both are less than in pure 0.55 m. NaCl — and the cilia cease soon.

Return to sea water. — With all of the above solutions recovery is at first (within half an hour) much greater than in the control. The 4 per cent solution is gradually destructive, and after four hours no revival was seen. With the 2 per cent solution recovery after five hours was decidedly greater than in the control. Weaker solutions also show well-marked protective action, as described in my former paper.

Anæsthetic concentration in sea water. — One per cent urethane produces only partial anæsthesia. In the 2 per cent solution anæsthesia is almost, and in 3 per cent quite, complete. Four per cent is toxic; the larvæ fail to revive after four hours' exposure, and later undergo disintegration.

Methyl urethane. — Comparatively few observations were made with this compound. Its action is weaker than that of ethyl urethane. In 5 vol. per cent solutions in 0.55 m. NaCl stimulation and loss of pigment are well marked, but the cilia are protected and remain active for about an hour. The 2 per cent solution shows an immediate action like that of pure 0.55 m. NaCl.

Return to sea water. — Both of the above solutions show well-marked antitoxic action, especially at first. The 5 per cent solution is fatal within four hours.

Anæsthetic concentration in sea water. — Two per cent solutions show no permanent anæsthetic action; and phototactic swarming, partly negative, is renewed in a few minutes; 5 per cent solutions anæsthetize the musculature, but the revival after four hours' exposure is imperfect.

Phenyl urethane. — Saturated solutions of this compound in 0.55 m. NaCl exhibit typical anti-stimulating and anti-cytolytic effects. There is almost no loss of pigment, and only slight immediate contraction, followed by instant relaxation; the cilia remain active. This solution, however, is somewhat too strong, and cytolysis sets in within two hours.

Return to sea water. — Recovery after thirty minutes' exposure is prompt and complete (much better than in the control). After cytolysis-

sis has begun in the solution the larvæ fail to revive in sea water. Observations with weaker solutions were not made.

Anæsthetic concentration in sea water. — Muscular anæsthesia is typical and complete in saturated solutions, but incomplete in half-saturated. The optimum is evidently intermediate; in saturated solutions the larvæ die within a few hours; in half-saturated they continue to live, but show slight contractions.

Hydrocarbons. — Experiments with chloroform, benzol, xylol, and toluol were briefly described in my former paper. The three cyclic hydrocarbons differ in their solubility in water, benzol being the most and xylol the least soluble; the physiological effects of their saturated solutions vary correspondingly; benzol produces complete reversible muscular anæsthesia in half-saturated solutions in sea water, and cytolysis in saturated; for toluol half-saturation is insufficient for anæsthesia, which, however, is typical in saturated; xylol in saturated solution produces only partial anæsthesia. In 0.55 m. NaCl all three fail to prevent immediate stimulation and loss of pigment, although they show well-marked antitoxic effects in concentrations corresponding to anæsthetic or slightly hypo-anæsthetic solutions in sea-water. Chloroform shows in NaCl solutions typical anti-stimulating and anti-cytolytic action in concentrations corresponding closely to those causing typical anæsthesia in sea water.

Observations were made last summer with three other substituted methanes, nitromethane, acetonitrile, and carbon tetrachloride.

Nitromethane (CH_3NO_2). — This compound shows highly characteristic action. In 5 vol. per cent solutions in 0.55 m. NaCl the larvæ contract strongly at first but without immediate loss of pigment; the cilia cease soon, and cytolytic action is evident within fifteen minutes. In 2.5 vol. per cent solution the initial contraction is moderate and followed by rapid relaxation and cessation of movement; there is no trace of pigment exit, and the cilia continue actively (in one experiment for some hours). In 1.5 vol. per cent solution there are prolonged contraction and well-marked loss of pigment, the cilia cease soon, and muscular contractions continue for a half-hour or more.

Return to sea water. — This is ineffective with the 5 vol. per cent solution after fifteen minutes; but if returned to sea water within five minutes the larvæ recover much more rapidly and completely than the control. Larvæ brought from 2.5 vol. per cent to sea water

within half an hour revive completely, and many show a return of phototaxis (both positive and negative); after three or more hours revival is less complete. In 1.5 per cent solutions the musculature and general organization are well protected for some hours, but not the cilia. The anæsthesia produced by nitromethane is very persistent, and revival in sea water is correspondingly gradual, but unusually complete if the exposure is not too prolonged.

Anæsthetic concentration in sea water. — Five vol. per cent solutions in sea water cause rapid cytolysis. In 2.5 vol. per cent anæsthesia is complete and normal.

Acetonitrile (methyl cyanide: CH_3CN). — This compound resembles nitromethane in its action, although the effective concentrations are higher. In 10 vol. per cent solutions cytolysis is rapid. In 5 vol. per cent solutions the immediate stimulation is slight, there is no loss of pigment, and the cilia remain active (for half an hour or more).

Return to sea water. — Recovery after half an hour in the 5 vol. per cent solution is much prompter and more complete than in the control, and many larvæ regain their phototaxis. After *ca.* three hours active contractions are renewed (much more active than in the control); after seventeen hours in the 5 per cent solution no revival was seen.

Anæsthetic concentration in sea water. — Anæsthesia is typical and complete in *ca.* 5 vol. per cent solutions, 10 vol. per cent solutions are rapidly destructive, and in 2.5 vol. per cent solution muscular anæsthesia is incomplete.

Carbon tetrachloride. — In its action this compound closely resembles benzol. In saturated solution in 0.55 m. NaCl it is quickly destructive. In one-half, one-third, and one-fourth saturated solutions immediate stimulation and loss of pigment are pronounced, as in the control; in the half-saturated solution ciliary movement is somewhat prolonged.

Return to sea water. — Well-marked protective action was observed in the one-third and one-fourth saturated solutions after seventeen hours' exposure; the half-saturated solution was fatal with this length of exposure, though it showed distinct protective action at first.

Anæsthetic concentration in sea water. — The saturated solution is quickly toxic. The half-saturated produces typical muscular anæsthesia, but causes some loss of pigment. In the one-third saturated solution anæsthesia is incomplete.

Phenanthrene and naphthalene. — These compounds are too insoluble to produce any appreciable effects in sodium chloride solution. The saturated solutions in sea water show little or no anæsthetic action;

the only evident effect is a disturbance of the heliotropic swarming which disappears in naphthalene-saturated sea water within a minute; later, however, a slight negative phototaxis shows itself. In sea water saturated with phenanthrene the normal heliotropic swarming continues almost unaffected at first, but later the sense of the response is largely reversed; within one hour about half of the larvæ show a negative phototaxis; the rest remain positive.

Chloral hydrate. — Observations with this compound were described in my former paper. Its action is apparently too gradual to prevent the immediate stimulation and loss of pigment, though in favorable concentrations it retards the disintegration caused by pure NaCl solutions and shows well-marked antitoxic action. In sea water muscular anæsthesia comes on gradually, but completely in solutions of 0.12 to 0.2 per cent; in 0.08 per cent there is partial inhibition of muscular movements, incomplete after eighteen hours. Concentrations of 0.5 per cent and upward are toxic.

Chloralose. — This compound is too insoluble to show well-marked action. In saturated solutions in 0.55 m. NaCl the initial stimulation and loss of pigment are similar to the control, and the cilia cease movement at once; muscular contractions continue for some time in this solution (an effect like that of other hypo-anæsthetic solutions), and there is a distinct general protective action.

Return to sea water. — Transfer to sea water after an hour in the above solution is followed by decidedly more complete revival than in the control; after twenty hours' exposure there was some revival in sea water, and the general disintegration was less than in the control.

Anæsthetic action in sea water. — In chloralose-saturated sea water the larvæ show normal heliotropic swarming at first; but a well-marked anæsthetic action asserts itself in time; in an hour the muscular contractions have almost ceased. Return to sea water after six hours is followed by complete revival.

Paraldehyde. — In 3 vol. per cent and 4 vol. per cent solutions in 0.55 m. NaCl the immediate stimulation and loss of pigment are prevented or greatly decreased, and ciliary breakdown is checked. Prolonged action of these solutions is injurious. The protective action is well marked at first (up to ten or fifteen minutes), but the larvæ die within an hour. No observations were made with weaker solutions. Anæsthesia in sea water is complete in 2.5 vol. per cent solutions; 5 vol. per cent is too concentrated and causes rapid cytolysis.

Acetanilide. — In saturated solutions of acetanilide in 0.55 m. NaCl there is only slight stimulation with almost no immediate loss of pig-

ment, and ciliary movement is prolonged. Within an hour cytolytic action sets in. Protection is well marked at first; larvæ transferred to sea water after ten minutes revive promptly and completely, many renewing their heliotropic swarming; after half an hour's exposure contractions also revive much more completely than in the control; but an hour's exposure is fatal. The saturated solution in sea water produces typical complete muscular anæsthesia, with continuance of ciliary movement. This concentration is higher than the optimum and causes injury or death with prolonged exposure.

Phenyl urea. — Saturated, three-fourths saturated, and half-saturated solutions of this compound in 0.55 m. NaCl fail to prevent the immediate stimulation and loss of pigment; in the first two concentrations ciliary movement is prolonged; in the half-saturated solution the cilia cease soon, and muscular contractions continue for some time (the usual effect of hypo-anæsthetic solutions).

Return to sea water. — Transfer to sea water after an hour in the above solutions is followed by more complete revival than in the control (best with the half-saturated solution). After eighteen hours' exposure no revival was seen.

Anæsthetic concentration in sea water. — The saturated solution causes contraction and loss of pigment followed by complete muscular anæsthesia. This solution is fatal within a few hours. In half-saturated solutions anæsthesia is incomplete.

Phenacetin and methacetin. — These compounds are too insoluble to influence the action of 0.55 m. NaCl. The saturated solutions in sea water have no apparent anæsthetic action; the larvæ show the usual strong positive phototaxis in such solutions; the only effect noted was an increased vigor of ciliary movement in the phenacetin solution.

III. GENERAL DISCUSSION.

On reviewing the above observations certain fundamental uniformities in the action of the different anæsthetics are seen. In the first place it appears that those strengths of solution which in sea water produce typical muscular anæsthesia are the most effective in preventing both the immediate strong muscular contraction in the sodium chloride solution and the immediate exit of pigment from the body cells. This parallelism is highly characteristic, and affords strong indication that the primary basis of both stimulating and pigment-

liberating actions is the same, namely, a rapid and well-marked increase in the permeability of the limiting membranes of the cells. In all those cases where the anæsthetic plainly increases the resistance of the pigment-containing cells to the permeability-increasing action of the salt solution it is found that the muscle cells also become resistant or irresponsive to its stimulating action. In the converse group of cases (*e. g.*, chloral hydrate, chloralose, phenyl urea, hydrocarbons) where the anæsthetic fails to prevent the immediate strong stimulation on contact with the solution — apparently because of a too gradual action — it also fails to prevent the exit of pigment. With most anæsthetics that produce prompt and typical anæsthesia both effects undergo a simultaneous and closely parallel decrease or prevention in solutions of appropriate concentration. Secondly, the general cytolytic or toxic action of the salt solution is also in all cases decreased or retarded in the presence of the anæsthetic, so that the larvæ on return to sea water recover their normal movements and behavior more completely than after a similar exposure to the pure solution. Substances of this class, in anæsthetic or moderately hypo-anæsthetic concentrations, thus exert a definite protective or antitoxic action; this is best marked in those cases where the initial stimulation and loss of pigment are entirely prevented, but it is shown also, though in general to a less degree, even where no such immediate antagonistic action is apparent.

The evidence at present available indicates that the anæsthetic produces these various effects by imparting to the plasma membranes or other surface structures (as cilia) an increased resistance to the alterative or disintegrative action of the pure salt solution. This it does presumably by altering the physical state of the lipoid components of these structures; the permeability-increasing and other associated effects ordinarily caused by the salt solution — including stimulation, and toxic action — are thus checked or prevented, for a time at least. It might be urged that the above observations contain no direct proof that changes in the permeability of the pigment-cell membranes form any index of similar changes in the membranes of the irritable elements. This may be admitted, although the conditions controlling the permeability of cells have been shown to be so widely uniform that any objection thus based seems rather formal than real. There is, however, independent evidence that the anæsthetic acts alike on the

pigment cells and on the contractile elements in the fact that it protects both simultaneously against the toxic or destructive action which the pure salt solution exerts on both. The initial action of the pure salt solution on pigment cells and on muscle cells thus appears to be essentially the same; and since clear evidence exists in the case of the pigment cells that this action consists in a marked and rapid increase of surface permeability, it is to be inferred that in the muscle cells also a similar change takes place. If the anæsthetic demonstrably prevents this rapid increase of permeability in the one case, it may be assumed to do so in the other. We infer, therefore, that it prevents stimulation and toxic action by preventing the rapid increase of permeability essential to both processes.

Increase of permeability in the pigment cells, as shown by exit of pigment, appears in the great majority of instances to be a reliable index of the occurrence of similar changes in the irritable elements. There is, however, even at this early stage, considerable differentiation among the cells of *Arenicola* larvæ, and the above rule appears to be not invariable; certain anæsthetics, *e. g.*, ethyl butyrate, ethyl valerianate, and chloroform in higher concentrations, extract considerable quantities of pigment in sea water, although causing anæsthesia without marked preliminary stimulation. A certain selective affinity of different cells for different anæsthetics seems thus already to exist in these organisms. The existence of such cases does not alter the general rule; the above substances, though attacking the pigment cells, show typical anti-cytolytic or protective — as well as anæsthetic — action on the muscle cells themselves. In the great majority of instances the degree of immediate exit of pigment in sodium chloride solutions containing anæsthetics — or antitoxic salts like calcium or magnesium chloride — exhibits a close parallelism with the intensity of the stimulating action on the musculature.

These observations have an obvious bearing on the general problem of the nature of the critical or primary change in the stimulation of irritable tissues in general. That a change of electrical polarization at the semi-permeable membranes of the irritable elements is the essential change in electrical stimulation has, since Nernst's study of this problem, been very generally recognized by physiologists. It seems improbable that a polarization change at the limiting membranes can be an exclusive peculiarity of the electrical form of stimulation:

other forms of stimulation ought theoretically to be associated with a similar effect, since all stimuli elicit the same essential response in an irritable tissue. If this inference is correct, there should be a physiologically produced change in the polarization of the limiting membranes during normal stimulation; and the electrical variation of stimulation is evidence that this is in fact the case. There is already wide agreement among general physiologists that the state of excitation is associated with a variation of electrical polarization at the limiting membranes. But apparently little agreement exists as yet with regard to the nature of the process that immediately conditions this change of polarization. The latter might conceivably be a chemical effect, due *e. g.* to an increased rate of oxidations, altering the ionic content of the protoplasm adjoining the membrane independently of alterations in the membrane itself; or it might be the expression of purely physical changes in the membrane, *e. g.*, changes of permeability, affecting directly its influence on ionic diffusion and so its polarization; or possibly both kinds of factors might be concerned. Any experimental evidence that the properties of the membrane are in fact altered during stimulation has thus an important bearing on the general problem of stimulation.

The above observations show that correlated with the loss of irritability is a definite alteration in the character of the limiting membranes. These become more resistant to changes in their permeability as the irritable tissue becomes more resistant to stimulation. This fact supports the view that in stimulation an increase in the permeability of the membranes is essential. Apparently normal irritability is associated with a certain state of the plasma membrane, in which the latter undergoes increase of permeability under certain conditions — especially changes in its electrical polarization — with a certain characteristic degree of readiness. During anæsthesia this readiness is decreased. The inexcitable condition produced by anæsthetics thus corresponds to one in which permeability is altered with difficulty.

Other facts and considerations, in part already well known, support this view. The most constant features of the stimulation process seem to be (1) the existence of a negative electrical variation, and (2) the existence of an inexcitable or so-called refractory period during a certain time after the beginning of the response. The time relations

of these two apparently quite different effects appear to correspond closely.⁸ The primary change in stimulation must therefore be of such a kind as to account for both. The only hypothesis — so far as I know — which gives a theoretically satisfactory explanation of this conjunction is that which refers *both* changes to a temporary and well-marked increase in the general permeability of the limiting membranes of the irritable elements. Such an increase would deprive the membranes of their capability of becoming the seat of an electrical polarization; it would therefore account for the observed fall in the potential difference between the interior and exterior of the irritable elements during stimulation, as well as for the inability of the tissue to respond to electrical excitation at this time, since this response depends (as Nernst and his successors have shown) on the production of polarization effects at the semi-permeable membranes, and during stimulation the necessary semi-permeability is lost. In addition to these theoretical grounds for regarding an increase of permeability as a necessary and primary condition of stimulation, there are various direct observations which point in the same direction. I have several times reviewed these observations and need refer to them here only briefly. Perhaps the most unequivocal evidence of this kind is the fact that in motile plant cells (of *Mimosa*, *Dionæa*, etc.) definite osmotic effects accompany stimulation, and indeed form the essential condition of the response. The turgor of these cells is temporarily lost; and since turgor depends on the semi-permeability of the plasma membranes, loss of turgor is a direct indication of loss of semi-permeability. No satisfactory substitute for this simple and adequate explanation has, to my knowledge, as yet been proposed. It is unlikely that the stimulation process differs in its essential nature in the two groups of organisms; and the above-described experiments with *Arenicola* larvæ indicate clearly that in the irritable tissues of animals the limiting membranes also undergo temporary increase in permeability during stimulation. There is also strong evidence that narcotic action in plants is dependent on a change in the character of the membranes identical with that just described. Anæsthetics prevent the characteristic response of *Mimosa* and plants with similar mechanisms; *i. e.*, the ether-impregnated plasma membranes no longer undergo rapid increase of permeability

⁸ Cf. TAIR: Quarterly journal of experimental physiology, 1910, iii, p. 221.

on stimulation, but retain their semi-permeability, so that turgor remains unaltered.⁹

The above-described protective or antitoxic action of anæsthetics resembles in all essential respects that of salts like calcium or magnesium chloride, and both seem primarily due to the influence of the antitoxic agent on the plasma membranes or other surface structures concerned (cilia). The suggestion that in antagonistic salt-actions the antitoxic salt acts by modifying the permeability of the membranes to the toxic salt, thus preventing the entrance of the latter into the protoplasm, has been made by several investigators,¹⁰ and lately

⁹ OSTERHOUT's observations on decrease in the electrical conductivity of fronds of the common kelp (*Laminaria*) under the influence of anæsthetics indicate that the plasma membranes undergo decrease in their general permeability during anæsthesia. Membranes so altered in irritable tissues would presumably undergo the marked permeability increase essential to stimulation — an increase sufficient for loss of semi-permeability, as shown by the conditions in *Mimosa* — with less readiness than normally. If a change in the electrical polarization of the membrane sufficient to cause this increase of permeability in the normal membrane no longer produced such an effect, the tissue would show itself inexcitable to stimulation. My own observations in *Arenicola* larvæ led me to infer several years ago that anæsthetic action was associated with a decrease of permeability. Cf. this Journal, 1909, xxiv, p. 30 seq.

¹⁰ The view that pure solutions of sodium chloride exert toxic action by abnormally increasing the permeability of the cell membranes, and that calcium and other antitoxic salts act by counteracting this effect, was first put forward by HERBST, in an extensive paper published early in 1904 (*Archiv für Entwicklungsmechanik*, xvii, p. 306). He describes experiments with young eels, similar to J. LOEB's earlier experiments with *Fundulus* (this Journal, 1900, iii, p. 331), and concludes that the injurious action of the pure NaCl solution on these animals is due largely to a permeability-increasing action on the respiratory epithelium of the gills, permitting undue entrance of the toxic salt; associated with this effect is a loosening of the union between the epithelial cells; both effects depend on an alteration of the surface membranes of the cells, and are prevented by the presence of calcium (cf. pp. 439-444). He regards other cases of antitoxic action of salts as probably an expression of similar surface changes (cf. also the section on the rôle of calcium, p. 476). G. N. STEWART in 1904 (*American yearbook of medicine and surgery, Medicine*, 1904, p. 527), at the end of a review of the important paper of LOEB and GIES on the antitoxic action of salts (*Archiv für die gesammte Physiologie*, 1902, xciii, p. 246) — in which the effects are referred to changes in the protoplasmic colloids, particularly the lipoids — writes: "The possibility ought to be taken into account that an apparent antitoxic action may sometimes, or in some part, be due to the production of a change in the permeabil-

considerable evidence has accumulated in its favor. The presumption until recently has always been that the toxic substance must first enter the cell protoplasm, and confusion has been introduced by a failure to take into account the properties of the plasma membranes. It has seemed necessary to many, in order to explain the physiological action of salts, to assume that these substances readily enter all cells; and this point of view was in fact definitely upheld by Loeb¹¹ and others, in opposition to Overton's contention that the plasma membrane, as an essentially semi-permeable structure, is in its unaltered state impermeable to neutral salts. If salts do not enter cells, how can they exert toxic or other physiological action? Overton¹² suggested that salts might affect intracellular processes by a purely superficial action, without entrance into the cell, but this point of view has hitherto received little attention.

Observations on *Arenicola* larvæ and sea-urchin eggs led me some years ago to the conclusion that what was essential in the toxic action of pure sodium chloride and similar solutions was *not the entrance of the salt into the cells*, but its general disintegrative or permeability-

ity of the cells to the toxic ions and not to an actual neutralization of their effects "within the cell protoplasm." This suggestion has an evident relation to his earlier expressed view that toxic agents like hæmolysins "act primarily by altering the permeability of the cell envelope" (*cf.* this Journal, 1903, ix, p. 80); inferentially, therefore, an antitoxic agent prevents this action. MATHEWS' experiments with *Fundulus* eggs in the summer of 1904 led him to conclude that changes in the permeability of the egg membranes played an essential part in the antitoxic action; thus the coagulative action of salts of cobalt, nickel, and manganese on the egg protoplasm was delayed in the presence of calcium. "We may conclude, therefore, that when calcium is present the cobalt, nickel, and manganese do not enter the egg, and accordingly the embryo is protected from them" (this Journal, 1905, xii, p. 441). Somewhat later LOEB also called attention to the possibility that salts like calcium chloride may prevent the toxic action of sodium chloride solution by altering the surface layer of the protoplasm, thus preventing the entrance of a toxic excess of the salt (*Archiv für die gesammte Physiologie*, 1905, cvii, p. 252), but without bringing forward specific evidence in support of this hypothesis. Recently he has reaffirmed this view, and his latest contribution to the subject (*Science*, N. S., 1912, xxxvi, p. 639) contains definite experimental evidence in its favor.

¹¹ *Cf.* J. LOEB: *Dynamics of living matter* (Macmillans, 1906, p. 41 *seq.*), and the article "Ueber physiologische Ionenwirkungen" in OPPENHEIMER's *Handbuch der Biochemie*, 1909, ii (1) p. 104.

¹² OVERTON: *Archiv für die gesammte Physiologie*, 1904, cv, p. 176.

increasing action on the plasma membranes.¹³ Prevention of this effect, as by the addition of calcium, thus involves antitoxic action. Various experiments were then cited supporting this contention. It was found, for instance, that if *Arenicola* larvæ are first treated for a few minutes with magnesium chloride the permeability-increasing action of subsequent exposure to pure sodium chloride solutions is prevented, and simultaneously the toxic action is greatly reduced.¹⁴ Alteration of surface permeability appears thus to be the essential in this form of toxic action. In general we must assign to the plasma membrane the significance of a diffusion-preventing and hence semi-permeable surface film, whose integrity is essential to the preservation of the normal composition of the protoplasm.¹⁵ Protection against the destruction of this semi-permeability by toxic agents is thus equivalent to antitoxic action.¹⁶ According to this point of view the semi-permeability of the plasma membrane is no merely incidental property, but one essential to the maintenance of the normal composition and properties of the cell; hence agencies or conditions that deprive the membranes of their semi-permeability are toxic, and in such cases the antitoxic agent works by counteracting this action.

Structures like membranes or cilia may be protected against breakdown in pure sodium chloride solution either by salts, as calcium or magnesium chloride, which presumably affect the total colloids of the membrane, or by organic anæsthetics, which act selectively on the lipoids. Antagonisms of this kind are thus not confined to antago-

¹³ Cf. my two papers in this Journal, 1909, xxiv, pp. 14 and 459; also 1910, xxvi, p. 106.

¹⁴ *Loc. cit.*, 1909; *cf.*, *e. g.*, p. 487; *cf.* also this Journal, 1911, xxvii, p. 296.

¹⁵ For a fuller discussion of this subject *cf.* Biological bulletin, 1909, xvii, p. 188; this Journal 1910, xxvi, p. 106. MACALLUM (Journal of the Royal Society of Canada, 3d Series, 1908, p. 145), ZANGGER (Ergebnisse der Physiologie, 1908, vii, p. 138 and elsewhere), and HOEBER (Physikalische Chemie der Zelle und der Gewebe, 3d ed. 1911, p. 252) have assigned a similar significance to the semi-permeability of the plasma membrane. Similar considerations apply to the membranes or other colloidal partitions inside the cell; these may thus play an important rôle in intracellular differentiation, as first suggested by HOFMEISTER in his "Chemische Organization der Zelle," Jena, 1901.

¹⁶ This interpretation of the antitoxic action of salts has been adopted by J. LOEB in his more recent discussions of this problem. *Cf.* Science, N. S., 1911, xxxiv, p. 653; Biochemische Zeitschrift, 1911, xxxvi, p. 277; 1912, xxxix, p. 194; xliii, p. 181.

nisms between ions, and the conception of equilibrated salt solutions, valuable as it is, is not broad enough to include the entire range of phenomena.¹⁷ The character and concentration of the ions in the protoplasm and medium are not the only factors determining the condition of the plasma membrane; the non-electrolytes, especially those which influence the state of the lipoids, are perhaps equally important. The above observations furnish direct evidence that the condition of the lipoids in the plasma membranes determines the rate and degree of action of toxic substances as well as of stimulating agencies. These phenomena have an evident relation to those cases where the addition of a lipoid to the medium influences toxic action — whether as activator or inhibitor; cholesterol in particular shows well-marked protective action in a variety of relations;¹⁸ it may protect blood corpuscles against hæmolytic agents; it also has been shown to increase the resistance of artificial lipid-impregnated membranes to alteration by similar agents.¹⁹ Apparently changes in the state of the lipoids already present in the membranes may have similar effects.

The precise rôle of the lipoids in the plasma membranes requires further research for its elucidation. Their condition in some way exerts a far-reaching control over cell processes. A relation to oxidations has been suggested by some investigators. According to Walde-mar Koch²⁰ it is possible that “phosphatides may, by means of their

¹⁷ LOEB and GIES were unable to obtain satisfactory evidence of antitoxic action between electrolytes and non-electrolytes (*Archiv für die gesammte Physiologie*, 1902, xciii, p. 246), and concluded that non-electrolytes differed fundamentally from electrolytes in their relation to this class of antagonistic action. It would appear, however, that non-electrolytes which influence lipoids form an exception to this rule. The state of these colloids is obviously influenced by other factors than the nature of the ions present.

¹⁸ ZANGGER gives a general discussion of these “antitoxic effects of lipoids especially of cholesterol” in a paper on “die Immunitäts-Reaktionen als physikalisches speziell als Colloid-Phänomen” in *Vierteljahrsschrift der naturforschenden Gesellschaft in Zürich*, 1908, liii, p. 408. Cf. also the article of LANDSTEINER in OPPENHEIMER'S *Handbuch der Biochemie*, Bd. ii (1), section on hæmolysis, pp. 444 *seq.*, for a summary of the chief facts, and literature.

¹⁹ PASCUCCI: *Beiträge zur chemischen Physiologie und Pathologie*, 1905, vi, p. 543.

²⁰ KOCH, W.: *Journal of pharmacology and experimental therapeutics*, 1910, ii, p. 265.

unstimulated cells play a rôle in excitations it is the summation of "issues for output." Certain facts of autonomic action show a certain harmony with this view. Loeb has recently shown in a number of cases that toxic action is more marked in the presence of output than in its absence or in presence of output. Apparently excitation processes are contributory or accessory to the destructive action in such cases.² If in these destructive excitations the participation of the Iqonites in the membranes is necessary, checking these excitations might prevent the toxic action by preventing alteration of the Iqonites thus preventing breakdown of the membrane. Similar considerations would, however, apply to any kind of toxic action in which excitations were a factor. Loeb's papers on this subject contain no evidence that he regards the membranes as the seat of the essential toxic action. Cyanoide do, however, check the permeability-increasing action of sodium salt solutions on echinoderm eggs, just as do Iqonid salts or calcium salts.³ This fact harmonizes with the supposition that excitation processes play a part in the breakdown of the membranes under certain kinds of toxic action and possibly the Iqonites — since their modification by anesthetics has a similar protective effect — are concerned in these excitations. Further discussion of this problem is best deferred until the accumulation of further data.

It must be recognized that the problem of the nature and conditions of toxic and autonomic action — whether of salts or of other substances — falls into the same essential category with the wider problem of the nature and conditions of physiological antagonisms in general. The relations actually existing in organisms between stimulation and inhibition — which are extremely opposite poles of the same process — call for consideration here. According to the outcome of the above experiments stimulation and inhibition under the influence of salts and anesthetics are — like the other two extreme kinds of these substances — dependent on changes in the state of the limiting membranes. I have already discussed the conditions of stimulation in some detail. The conditions of the other process of inhibition in the animal organism are probably fundamentally similar to those of artificial inhibition under the influence of anesthetic salts or the electric current (anesthetics). In this view

² Loeb, *J. Biochemische Gesellschaft*, 1910, vol. 3, pp. 100 and 101.

³ Loeb, *J. this Journal*, 1910, vol. 3, p. 2.

inhibition is the expression of a change in the state of the plasma membranes, opposite in kind to that associated with stimulation; *i. e.*, the membrane is rendered by inhibition less susceptible than normally to permeability increase and hence to stimulation. Exactly how this occurs is as yet unknown. If, however, stimulation is the expression of a change of electrical polarization in the one direction (depolarization), a polarization change in the opposite direction is probably concerned in inhibition. In the reflexes of higher animals excitation of one set of motor neurones involves the inhibition of the antagonistic set, as Sherrington has shown, though the conditions of this interference remain unexplained. *An electrotonic influence of one set of neurones on the other* seems the most likely basis of such an effect; and this interpretation is supported by certain phenomena of galvanotropism in which the orienting or directive influence of the external electric current depends on a simultaneous inhibition and reinforcement of opposite motor innervations which are undoubtedly of central origin.²³ Sherrington has put forward the hypothesis that alterations in the state of the membranes at the synaptic junctions are a condition of inhibition,²⁴ an explanation plainly consistent with the general point of view advocated in this

²³ Cf. the observations of LOEB and GARREY on *Amblystoma* (Archiv für die gesammte Physiologie, 1896, lxx, p. 41), and LOEB and MAXWELL (*Ibid.*, 1896, lxxiii, p. 121) on *Palæmonetes*.

That is, external electrical conditions that favor the activity of one set of neurones inhibit the activity of the antagonistic set. Similarly, the electrical variations that *normally* accompany the activity of, *e. g.*, the flexor neurones may be incompatible with — or, in other words, may inhibit — the activity of the extensor neurones. Neurones so related would be capable of alternating but not simultaneous activity. I venture this suggestion here as possibly throwing light on the mechanism of a very obscure process. From the above observations LOEB concluded "that the nervous elements of the flexors and extensors in the central nervous system which are affected by the ions (in galvanotropism) possess an opposite orientation," such that an electrical current which produces catelectrotonus (or excitation) in one causes anelectrotonus (or inhibition) in the other. Cf. his Comparative physiology of the brain, p. 162, and the discussion following, also the above papers. He does not, however, so far as I am aware, consider the case of reciprocal inhibition. The structural conditions in the central nervous system may quite possibly be such that the action current of one neurone instead of stimulating *inhibits* the activity of the adjoining antagonistic neurone.

²⁴ SHERRINGTON: Integrative action of nervous system, p. 192.

paper. The conditions of mechanical inhibition in the ctenophore swimming plate suggest that the membrane of the inhibited tissue becomes increasingly resistant to permeability change,²⁵ and in the case of cells like motor neurones the conditions are probably essentially similar. It is highly desirable to reach, if possible, a valid unitary point of view from which to consider these and related phenomena; its attainment would greatly facilitate effective and well-directed research; and the indications are already strong that the conception of the plasma membrane as a structure whose condition exerts a controlling influence over cell processes — largely through changes of electrical polarization conditioned by changes of permeability — will furnish this point of view.

SUMMARY.

1. In the action of pure isotonic NaCl solutions on *Arenicola* larvæ the most evident effects are: (1) strong stimulation of the musculature, causing intense and prolonged contraction, (2) increase in the permeability of the pigment-cell membranes sufficient to allow visible exit of pigment, (3) immediate arrest of ciliary movement, followed by disintegration of the cilia, and (4) a general toxic action.

2. In the presence of a large number of anæsthetics, in concentrations corresponding to those producing typical neuromuscular anæsthesia in sea water, all of these characteristic immediate effects of the pure salt solution are diminished or prevented.

3. In general, the permeability-increasing action and the stimulating action of the salt solution undergo closely parallel decrease or prevention in the presence of the anæsthetic. Prevention of sudden permeability increase thus seems equivalent to prevention of stimulation; it is also equivalent to prevention of the immediate toxic action of the solution. The anti-stimulating and the anti-cytolytic effects of the anæsthetic thus show a definite parallelism.

4. The conclusion is drawn that in anæsthesia the essential effect is a temporary alteration in the condition of the surface films or plasma membranes of the irritable elements, of such a kind that these membranes no longer undergo, under the usual conditions of stimulation, the rapid increase of permeability essential to this process.

²⁵ LILLIE, R.: this Journal, 1908, xxi, p. 200; cf. p. 215 seq.

5. The membranes thus become during anæsthesia increasingly resistant to permeability-increasing agencies: this involves increased resistance to those forms of toxic action which depend on destruction of the normal semi-permeability of the membranes. Hence the association of an anti-cytolytic or antitoxic action with the anti-stimulating action of the anæsthetic. These observations also indicate that the degree of resistance of the membranes, and of other colloidal structures like cilia, is intimately dependent on the state of their component lipoids.

THE CONDITIONS DETERMINING THE VOLUME OF THE ARTERIAL BLOOD STREAM.

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I. THE HEART AS THE METRE OF THE CIRCULATION.

THE conditions determining the circulation rate, that is, the volume of the arterial blood stream, present a problem of extraordinary significance. This is, in fact, the essential problem of the circulation. Harvey solved it qualitatively. A number of modern investigators have formed more or less crude estimates of the volumes involved. Henderson has attempted to express it in a quantitative law.

The heart is both a pump and a metre. The blood stream in any unit of time (*e. g.*, one minute) is the product of the number of heart beats, that is, the pulse rate, multiplied by the volume discharged by a single stroke of the left ventricle. As the pulse rate is always easily determined, the problem of the volume of the blood stream reduces itself to the problem of the systolic discharge.

Upon this question investigators are sharply divided. Henderson,¹ Bohr,² and Pütter³ hold that the systolic discharge under normal conditions and at slow rates of beat is for the individual a practically unvarying quantity. Accordingly at such rates the blood stream must vary in proportion to the pulse rate. At more rapid rates, as Henderson has shown, one beat follows another before the ventricle has had time to relax sufficiently to receive a full charge of blood. The systolic discharge is thus diminished, and although the volume of the blood stream still increases as the pulse becomes more rapid, it falls further and further behind proportionally.

¹ HENDERSON, Y.: this Journal, 1909, xxiii, p. 345. For a general review of the literature see TIGERSTEDT, R: *Ergebnisse der Physiologie*, 1905, iv, p. 487.

² BOHR: *Skandinavisches Archiv für Physiologie*, 1909, xxii, p. 221.

³ PÜTTER: *Zeitschrift für klinische Medizin*, 1911, lxxiii, p. 342.

This conception of the relation of the pulse rate to the systolic discharge receives powerful support from observations upon the size of the human heart at various rates of beat by means of the X-ray.⁴ Such observations have shown decrease in volume with increase in rate of beat in a relation closely similar to those variations which Henderson recorded plethysmographically, and expressed diagrammatically as the combined result of increasing tonus and decreasing amplitude of stroke.

On the other hand, Zuntz⁵ and his adherents hold that the heart is capable of varying the amplitude of its stroke enormously independently of the rate of beat. During physical exercise, according to this view, not only is the pulse rate accelerated, but the systolic discharge is also augmented to several times its resting value. Such an increase of the systolic discharge would necessarily involve a corresponding increase in the diastolic filling of the ventricle. No sufficient evidence has been adduced, however, to show *how* it is possible for the heart to increase the rapidity and extent of its relaxations during the abbreviated diastoles of a rapid pulse rate. It is the heart's own inherent rate and extent of relaxation which, more than any other factor, determines the volume of the tidal blood. Certainly the heart cannot for any great while throw out more blood during systoles than runs into it during diastoles.

In support of the theory of the variability of the systolic discharge Plesch⁶ has recently pointed out that in the heart beat we must recognize (1) a residual volume, *i. e.*, the blood remaining in the ventricles at the end of systole, (2) a tidal volume, *i. e.*, the diastolic inflow and systolic discharge, and (3) a complemental volume, *i. e.*, the increased quantity of blood which the heart may take in during extreme (vagus) relaxation. He considers that the tidal volume may increase at the expense of the complemental and residual volumes in much the same manner as occurs in respiration. He overlooks the fact, however, that this matter was carefully investigated by Henderson, who found that no increase of this character ever occurs. On the contrary, the latter concluded that in the normal heart, in spite of the extensive tonus changes occurring at various rates of beat, the volume

⁴ DIETLEN: *Ergebnisse der Physiologie*, 1910, x, p. 598.

⁵ ZUNTZ: *Zeitschrift für klinische Medizin*, 1912, lxxiv, Nos. 3 and 4.

⁶ PLESCH: *Zentralblatt für Physiologie*, 1912, xxvi, p. 90.

of the systolic discharge is a logarithmic function of the duration of diastole, which in turn is a function of the pulse rate.

There are two, and apparently only two, elements in the normal control of the heart which might conceivably induce such a variability of behavior as Zuntz and Plesch assume, — venous pressure and the extrinsic cardiac nerves, vagus and accelerator. It is well known that these nerves exert a powerful influence upon the amplitude of beat. It is very questionable, however, whether during normal life this influence is ever manifested independently of variations in the rate of beat. In fact, Henderson has shown that under experimental conditions as near normal as possible tonus, rate, and amplitude of beat always maintain mutually dependent relations. In spite of this Zuntz points out that on stimulation of the vagus the exposed heart exhibits a great increase in the volume of its strokes, and fails to recognize that this effect is always associated with, and according to Henderson's conception is due to, slowing of the rate of beat and prolongation of diastole.

The other element which may influence the extent to which the heart is filled during diastole and the volume which it discharges during systole is venous pressure. Zuntz quotes the observations of Braune⁷ to show that the venous stream to the right heart is accelerated by muscular movements; and Hooker,⁸ in fact, in his observations upon a man exercising upon a stationary bicycle found that venous pressure is increased. Hooker, however, considers that this rise of venous pressure is probably due to a damming back of blood from the right heart owing to a *decrease* in the amplitude of the strokes of the ventricle accompanying the increase in rate of beat. Zuntz assumes that the increased respiratory movements of physical exercise tend to assist the circulation by aspirating blood toward the thorax. This influence, however, cannot reach more than a few millimetres outside the thorax, since suction cannot be transmitted to any distance over collapsible vessels.

Krogh⁹ has recently discussed this topic partly on the basis of Henderson's volume curves, and reaches the conclusion that venous pres-

⁷ BRAUNE: Berichte der sächsische Gesellschaft der Wissenschaften, 1870, xxii, p. 261.

⁸ HOOKER: this Journal, 1911, xxviii, p. 235.

⁹ KROGH: Skandinavisches Archiv für Physiologie, 1912, xxvii, pp. 126 and 227.

sure determines the extent of the diastolic filling of the right ventricle, and is thus the principal factor controlling the volume of the arterial blood stream. Krogh tacitly assumes one point which we wish expressly to emphasize, namely, that if during normal life the systolic discharge varies independently of the rate of beat, such variations must be regulated by some condition outside the heart itself, probably some veno-pressor mechanism. Otherwise the conception of the circulation held by Zuntz and his school would involve the assumption that the heart is itself capable of varying the amplitude of its contractions to a degree equal to that exhibited by skeletal muscle. In fact, however, the particular characteristic of cardiac tissue in contrast with ordinary muscle is the All or None law.

From the foregoing discussion it will be seen that the problem of the circulation rate resolves itself into the question of the conditions determining the systolic discharge, and that this question in turn resolves itself into two subsidiary questions: (1) Are the vagus and accelerator nerves capable of inducing variations in amplitude of beat independently of rate? And (2) are such variations induced by alterations in venous pressure? There is, of course, ample evidence to show that when the heart has been excised and is beating under artificial conditions the answer to both these questions is in the affirmative. The problem which we have set ourselves in this and subsequent papers, however, is to determine, not what the heart *may* do under abnormal conditions, but what it *actually does* under conditions as nearly normal as experimental requirements will permit.

II. THE PLETHYSMOGRAPHIC METHOD OF RECORDING THE BLOOD STREAM.

Our experiments were carried out upon cats and dogs. The former were in some cases etherized, in others decapitated by Sherrington's method; the latter, after an initial injection of morphin, were etherized. For the cats artificial respiration was supplied; for the dogs natural respiration of compressed air (6 to 10 cm. of water) with an arrangement for more or less rebreathing. Arterial pressure was recorded by means of a Hürthle manometer (old style) connected with a cannula in the carotid artery. The thorax was opened by cutting

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all of the costal cartilages. The mammary arteries were ligated or clamped. The pericardial sac was slit and a plethysmograph of the form described by Henderson¹⁰ was placed over the ventricles so that a rubber curtain covering the window of the plethysmograph rested in the auriculo-ventricular groove. Care was taken that it should fit accurately so as to avoid any leak or any considerable compression of the atrio-ventricular orifices. Attention was also paid to supporting the plethysmograph so that the heart should not move in and out through the window. Neglect of this precaution results in a distortion of the volume curve, for auricular systole tends to lift the ventricles and to push them further into the plethysmograph. It thus exaggerates the influence of the auricular contraction upon the filling of the ventricles.¹¹

The credit of being the first to use this method is generally assigned to Tigerstedt. In fact, however, it was first employed by François-Frank.¹² As far back as 1877 he published volume curves of correct form and even applied the method to the human heart. Tigerstedt,¹³ after using the method for a time, concluded that it was unsuited to quantitative investigations. The prevalence of this opinion has prevented a more general use of it. This is particularly unfortunate, as it is an easy, direct, and illuminating procedure for the study of the behavior of the heart under conditions relatively little altered from those of normal life. Improper adjustment or support of the plethysmograph may indeed easily lead to extremely erroneous curves; *but it has been shown by Rothberger,¹⁴ by a comparison of simultaneous volume curves and stromuhr records, that, when care is taken to avoid such errors, the method is one of a fair degree of quantitative accuracy.*

The volume changes of the ventricles were recorded by large tambours (12 cm. in diameter for dogs and 10 cm. for cats) connected to the plethysmograph by large bore rubber tubing (100 cm. length and 9.5 mm. and 8 mm. interior diameter, respectively). The question has

¹⁰ HENDERSON, Y.: this Journal, 1906, xvi, p. 335.

¹¹ Cf. MÜLLER and FINCKH: Zeitschrift für experimentelle Pathologie und Therapie, 1912, xi, p. 266.

¹² FRANÇOIS-FRANK: Travaux du laboratoire de Marcy, 1877, iii, p. 321; also Archives de physiologie, 1890, p. 395.

¹³ TIGERSTEDT: Ergebnisse der Physiologie, 1907, Jrg. vi, p. 273.

¹⁴ ROTHBERGER: Archiv für die gesammte Physiologie, 1907, cxviii, p. 353.

been raised (Hermann Straub¹⁵) whether such a tambour is capable of recording with accuracy large and rapid volume changes. It needs no mathematics to show that a tambour over which the rubber is stretched is utterly unreliable as a volume recorder. When Marey covered a tambour, he did not tie the rubber tightly over the lip, as is frequently done nowadays, but held it in place by a ring pressed down upon (not around) the edge of the bowl. In his instruments the rubber was not stretched. A considerable volume change was thus possible without any appreciable change in the pressure within the tambour. We have gone further and have arranged that this rubber sheet

should always be slack. Our tambours have a flat edge 5 cm. wide. This is heated and coated with melted beeswax. The sheet of rubber (dental dam) is spread loose upon a table, and the tambour is pressed down upon it and held until the beeswax hardens. The free edges of the sheet of rubber are then tied down into the groove running around the tambour. The round aluminum float of the tambour (with a diameter four fifths as great as that of the tambour itself) is next coated with melted beeswax and pressed down upon the centre of the sheet of rubber, which is thus stretched down 4 to 6 mm. The float is held in this position until the beeswax hardens. When it is then released, the sheet rubber shows a certain amount of slack all around its edges. This is sufficient to allow the float to move through a distance of 10 mm. for the large instrument and 6 mm. for the small without putting the slightest tension upon the rubber (see Fig.

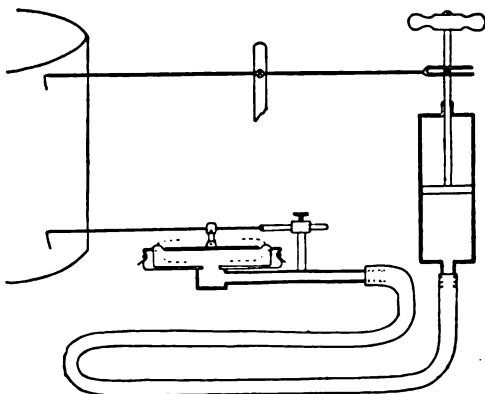


FIGURE 1. — Showing arrangement of tambour used to record the cardiac volume curves, and method of testing by means of a syringe. Note the looseness of the sheet of rubber between the edge of the float and the rim of the tambour. The dotted lines in the tambour indicate the extent of movement possible without stretching the rubber in the least.

¹⁵ STRAUB, H.: *Journal of physiology*, 1910, xl, p. 378; for HENDERSON's reply see *Archiv für die gesammte Physiologie*, 1912, cxlvii, p. 111; also HENDERSON and JOHNSON: *Heart*, 1912, iv, pp. 77-79.

1). The tambours were used entirely within the range of volume thus allowed, namely, 80 c.c. for the large instrument and 25 c.c. for the small. The pressure within the large tambour was found to be + 2.5 mm. of water at the lowest point of the stroke and + 2.8 mm. at the highest. The ability of the tambours to record accurately large and rapid volume changes was tested by attaching each by the

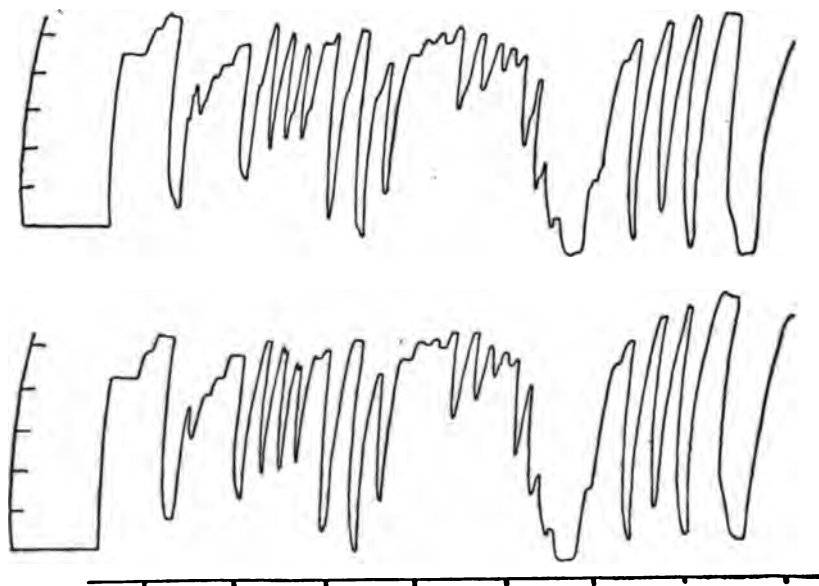


FIGURE 2. — One third the original size. Showing result of test of tambour. These curves were recorded simultaneously by a stylus attached to the plunger of a syringe (upper curve) and by the tambour (lower curve). Time in seconds. For arrangement see Fig. 1.

rubber tubing used with it to a syringe the plunger of which was rapidly thrust in and out. In Fig. 2 the upper curve was recorded by a stylus attached to the plunger of the syringe (with a stroke volume of 70 c.c.) and the lower record by the larger of the two tambours. It will be seen that the lag and inertia of the instrument were entirely negligible. After trying practically all forms of volume recorders that have as yet been invented, we believe that none of those affording direct graphic records (*i. e.*, not involving photography) compare in accuracy with a large tambour arranged in the special manner here described.

In all of the investigations on the volume curves made in this laboratory during the past nine years tambours of this type have been used exclusively.

III. THE INFLUENCE OF THE VAGUS UPON THE SYSTOLIC DISCHARGE.

Experiments to show the influence of the vagus upon the amplitude of the heart beat and the form of the volume curve were carried out upon five dogs and twelve cats. In the former the nerve on only one side of the neck was cut and the peripheral end stimulated; in the latter the nerve was stimulated in some cases in the neck and in

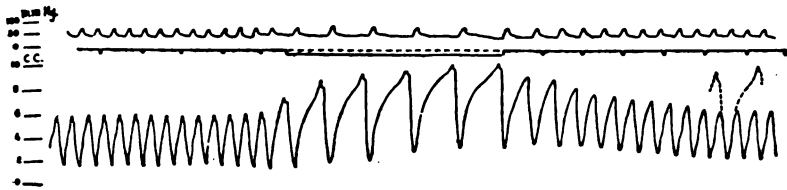


FIGURE 3. — One third the original size. From a decapitated cat. Upper record arterial pressure pulse. Base line shows time in seconds and duration of vagus stimulation. In the volume curve systole is expressed as a down-stroke and diastole as an up-stroke. The dotted lines at the right show one of the large vagus beats superimposed upon one of less amplitude. From the exact agreement — the smaller consisting merely of shorter arcs of the larger — it follows that vagus stimulation increases the systole discharge because, and solely because, it prolongs diastole and thus allows a more complete relaxation and filling of the ventricles.

others within the thorax, where (in cats) a more purely inhibitory effect is obtained. The strength of stimulus was adjusted so as to cause a distinct slowing of the rate but not so as to stop the heart altogether. When a stimulus too weak to affect the rate was tried, no effect whatever was obtained. *In other words, no alteration of amplitude without a corresponding change of rate is inducible through the vagus.*

The results of these experiments without exception¹⁶ were of the type shown in Fig. 3. Examination of the volume curve shows that the beats during vagus stimulation were much larger than those before and afterward, but that this increase was not a primary effect. It was dependent upon the slowing of the rate of beat. The easiest way to see this is to imagine one of the large slow beats in the volume curve to be placed over one of the small quick beats, so that the

¹⁶ For similar volume curves see HENDERSON: this Journal, 1906, xvi, p. 349; and 1909, xxiii, p. 399; also STRAUB, H.: Journal of physiology, 1910, xl, p. 387. Cf. DE HEER, Archiv für die gesammte Physiologie, 1912, cxlviii, p. 1.

lower ends of the curves correspond, — just as in geometry it is customary to compare angles and triangles by their superimposability. At the right in Fig. 3 the dotted lines show how the larger beat would lie upon the smaller.¹⁷ From the exact correspondence of these two beats, so far as the smaller extends, it is evident that the vagus influence does not alter the heart's contraction and relaxation, *i. e.*, the down-stroke and the up-stroke in the volume curve. It merely slows the rate at which the beats follow each other. By prolonging diastole it affords time for a more complete relaxation, which in turn provides the necessary condition (*i. e.*, more complete filling of the ventricle) for the succeeding large contraction.

The whole matter may be summarized as follows: When the heart is beating normally and with an adequate venous supply, the ventricles contract and relax in curves of characteristic and practically unvarying (*i. e.*, superimposable) form. At rapid rates of beat the volume curve consists of a series of short arcs cut off the lower ends of these curves. At slow rates the arcs are correspondingly fuller. In other words, *the vagus influence is not capable of causing the heart to deviate from the law*¹⁸ *that the volume of any systolic discharge is the ordinate of that point in the relaxation curve of the ventricles for which the abscissa is the duration of the preceding diastole.*

Mention should be made of the great decrease of tonus (indicated by a rise of the volume curve as a whole) which the vagus may induce in the ventricle. Thus we have curves in which during vagus stimulation the heart increased in size so greatly that in spite of the amplitude of the slow full beats the ventricles contained more blood at the end of systole than was held by them at the end of diastole when the rate of beat was rapid, tonus high, and the ventricles therefore small. Such tonus changes do not, however, alter to any considerable extent the form (*i. e.*, the superimposability) of the volume curve. The amplitude (*i. e.*, the tidal volume, or systolic discharge) is determined by the rate of beat and duration of diastole. In estimating the volume of the arterial blood stream per minute from the form of the volume curve it is not necessary to take into account the tonus changes of the heart.

¹⁷ For a more complete expression of this superimposability, see HENDERSON: this Journal, 1909, xxiii, p. 354.

¹⁸ HENDERSON, Y.: The law of the systolic discharge, International Physiological Congress, Vienna, 1910.

IV. THE INFLUENCE OF THE ACCELERATOR NERVES.

In five dogs the annulus of Vieussens and the stellate ganglion were stimulated. In ten cats not only were these structures stimulated on both sides, but every strand of fibres leading from the sympathetic ganglia to the heart was carefully dissected out and the effect of its stimulation upon the volume curve recorded. In no case were the effects of even the strongest stimulations at all comparable in intensity to those (of opposite character) obtained from the vagus. In a few cases no effect whatever upon the heart's rate or amplitude of beat was produced. In comparison with the inhibitory control by the vagus the accelerator influence appears to be an element of rela-

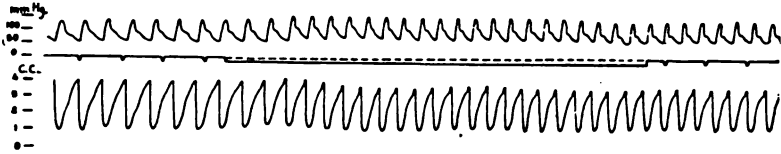


FIGURE 4. — One third the original size. From a decapitated cat. Stimulation of left stellate ganglion. Otherwise all arrangements were similar to Fig. 3 (*q. v.*). Note that the quicker rate decreases the amplitude. The volume curves at the more rapid rate are merely shorter arcs of the slower and fuller beats. Thus the duration of diastole determines the volume of the systolic discharge.

tively slight potency in the regulation of the heart. The most marked results were obtained from stimulation of the stellate ganglion itself. In different experiments, and in some cases at different periods of the same experiment, two distinct types of reaction were obtained.

The reaction in all cases in which the heart was beating with approximately normal vigor and in which the arterial pressure was nearly normal is illustrated in Fig. 4. The essential feature was a distinct acceleration of the rate of beat. This was accompanied by a corresponding decrease in the amplitude of beat, so that the superimposability of the volume curves of the different rates and amplitudes was accurately maintained. *They were simply longer or shorter arcs of an identical curve.* Accordingly, just as in vagus stimulation, the duration of diastole determined the volume of the tidal blood and the systolic discharges.

In the other type of reaction the characteristic feature was an in-

crease in the amplitude of stroke, while the rate of beat remained unaltered or even increased. In other words, an *augmentor* effect, in contradistinction to the merely accelerator influence discussed in the previous paragraph, was obtained. An example of such augmentation is shown in Fig. 5. Now it is one of the postulates of the law of the systole discharge that there is in reality no "augmentor" nerve to the normal mammalian heart. Proof that such an influence occurs under normal conditions would afford a refutation of the law. The only claim made for this law, however, is that it holds good for normal conditions of the circulation with vigorous heart action. Under abnormal conditions the heart deviates from it. Accordingly it is important to note that *this augmentor effect was never obtained except when the heart was beating with considerably diminished vigor and arterial pressure was low.*

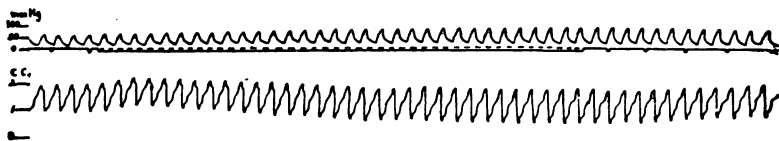


FIGURE 5. — One third the original size. Decapitated cat. Arrangements as in Fig 4. During stimulation of the stellate ganglion the heart rate remained unaltered, while the amplitude of stroke increased. Note, however, that the heart was much below normal vigor and arterial pressure was very low. It was only under such conditions that an augmentor effect such as is here shown was obtained. Under more normal conditions the effect was always acceleration and decreased amplitude of stroke, as in Fig. 4.

The augmentor effect was at most a small matter. It never exceeded 30 per cent of the amplitude previous to stimulation. It appears incapable of causing a supernormal amplitude of stroke, for in none of our experiments did it suffice to restore even a normal amplitude. Its insignificance as a factor in determining the volume of the systolic discharge is emphasized by the fact that the conception of the circulation held by Zuntz and his adherents requires the production in some manner of an augmentation of the systolic discharge of 100 to 200 per cent simultaneously with a great acceleration in the rate of beat. As the augmentor effect occurred only in feebly beating hearts we feel justified in concluding that it is merely a modification of the accelerator influence, and that when the heart is generating excitations of full strength this influence has solely the function of quicken-

ing their rate. It is only when the excitations are subnormal that the accelerator influence can appear as augmentation.

We have not thought it necessary for the objects of this paper to study the effects upon the volume curve of simultaneous vagus and accelerator stimulation. Rothberger and Winterberg¹⁹ have shown that such excitation is prone to throw the heart into fibrillation. It is thus excluded from among the possible normal modes of adjustment of the heart to the requirements of the circulation.

V. CONCLUSIONS.

The volume of the arterial blood stream at any time is equal to the pulse rate multiplied by the volume discharged by a single stroke of the left ventricle.

The systolic discharge under normal conditions (*i. e.*, with an adequate venous pressure and supply) and at slow rates of beat is for the individual a practically unvarying volume.

At more rapid rates the abbreviation of diastole cuts short the relaxation of the ventricle, and diminishes the quantity of blood received, thus reducing also the volume thrown out by systole.

Stimulation of the vagus may increase the amplitude of stroke very considerably, but this effect is wholly dependent upon the slowing of the rate of beat and lengthening of diastole. The blood stream is decreased.

Stimulation of the accelerator nerves, when the heart is beating with a fair degree of vigor, quickens the rate of beat and decreases the amplitude because of the shortening of diastole. An augmentor effect is not obtained except when the heart is beating feebly.

From these observations we conclude that the vagus and accelerator are incapable of altering the amplitude of stroke independently of the rate of beat. Under normal conditions these nerves influence primarily the rate alone. The time which the rate of beat allows for the diastolic relaxations determines the volume of the systolic discharges.

In later papers we shall report experiments upon the relation of venous pressure to cardiac efficiency and the influence of respiration upon the blood stream.

¹⁹ ROTHBERGER AND WINTERBERG: *Archiv für die gesammte Physiologie*, 1911, cxli, p. 343.

VARIATIONS IN THE SENSORY THRESHOLD FOR FARADIC STIMULATION IN NORMAL HUMAN SUBJECTS.
— I. THE DIURNAL RHYTHM.

BY G. P. GRABFIELD AND E. G. MARTIN.

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THE method devised by one of us¹ for measuring induction shocks has proven to afford a particularly satisfactory means of estimating the sensitiveness of the human receptive mechanism.² Such determinations of the sensory threshold promise to be of value in neurological work, and in allied branches of medical investigation. In anticipation of such a function for them, we have undertaken a series of studies of the variations in sensory threshold which may occur in normal individuals. Some of the possible normal sources of variation in sensory threshold are: a diurnal rhythm, such as is reported by Ostanikow and Gran³ for reaction time, and by Lombard⁴ for ergographic fatigue; a variation according to age, such as is known to exist for heart rate, respiratory rate, and similar functions nervously controlled; a variation according to sex; and variations accompanying habits, such as hours of eating or sleeping, amount of exercise taken, use of tobacco and alcohol.

This paper deals with the first of these possible sources of variation, our problem being the solution of the following question: Is there a constant diurnal variation in the receptive mechanism of the body, as indicated by the sensory threshold for faradic stimulation, and, if so, what relation does it bear to diurnal variations in other bodily conditions?

¹ MARTIN: this Journal, 1908, xxii, p. 116, and 1910, xxvii, p. 226; also *The measurement of induction shocks*, New York, 1912.

² MARTIN, E. G., PORTER, E. L., and NICE, L. B.: Psychological review (in press).

³ OSTANIKOW and GRAN: *Neurologische Centralblatt*, 1893.

⁴ LOMBARD: *Journal of physiology*, 1892, xiii, p. 25.

Variations in the Sensory Threshold for Faradic Stimulation. 301

Method of measuring stimulation strength. — The strengths of stimuli are measured in β units.⁵ For determining these units the following data are required: (a) the primary current, corrected, if necessary for core magnetization;⁶ (b) the secondary positions at which threshold stimuli are given, (1) with the tissue only in the secondary circuit; (2) with an additional resistance of 10,000 ohms in circuit with the tissue; (3) with an additional resistance of 20,000 ohms in circuit; (4) with an additional resistance of 30,000 or 40,000 ohms in circuit; (c) the resistance of the tissue. The results were tabulated on separate cards as follows:

NAME, JONES. TIME, 10.30 A. M. DATE, 6/13/12. No. 3.

Pri. cur. 0.4	Tissue	10,000	20,000	30,000	Average
Sec. pos. . . .	18.9	15.9	14.9	13.5	. . .
<i>M/L</i>	287	626.5	905.5	1595	. . .
<i>Z</i>	124.7	268	394	694	. . .
<i>A</i>	5400	6000	6300	5900
β	80.0	82.3	79.6	83.3	81.3
<i>R</i> {	Known . .	2000	1000	1500	<i>R'</i> . . .
	Unknown .	1900	2100	1800	1400
					1900

The name of the subject, the time of the observations, the date, and the number of the particular observation in the series of that date were placed at the top of the card. The primary current (Pri. cur.) was in every case 0.4 ampere. The vertical columns represent the readings when the resistance of the tissue alone was in the secondary circuit, and when the additional resistances were placed in the secondary circuit. The horizontal column headed secondary position (Sec. pos.) indicates the position where the secondary delivers the threshold shock. M/L ⁷ is obtained from a table for this inductorium,

⁵ MARTIN: this Journal, 1910, xxvii, p. 230; also The measurement of induction shocks, p. 76.

⁶ MARTIN: The measurement of induction shocks, p. 46.

⁷ MARTIN: *Loc. cit.*, p. 55.

Coil B. I_c is the value of the primary current expressed in amperes, as corrected by the formula for core magnetization:

$$I_c = I \times (I + KI)$$

where I represents the current as read from the ammeter and K a constant. For Coil B the value of K is 0.22. When 0.4 ampere are used, I_c becomes 0.435 ampere. Z is a quantity found by the formula:

$$Z = M/L \times I_c$$

and represents the intensity of the stimulus used uncorrected for the resistance of the secondary circuit. The method gives four different values of Z with four different secondary resistances. To get a rough estimate of the value of β (the absolute threshold value) and as a check on the observations of secondary position, we plotted the Z 's against the resistances. The four points thus determined should be in a straight line, since "the curve of increasing stimulus against increasing resistance is a straight line" (Martin). The Z 's were plotted as abscissæ, and the point where the straight line through the four points thus determined crosses the ordinate for zero resistance gives the value of β . If any of the four points was more than 10 per cent away from the straight line, it was discarded. The formula for computing β is

$$\beta = \frac{AZ}{R + A}$$

A is a purely mathematical factor found by the formula

$$A = \frac{ZR' - Z'R}{Z' - Z}$$

From the four values of Z we get three values of A , using for the quantity Z in the formula the Z found in the tissue column and for R the tissue resistance in every case, and varying the Z' and R' by substituting for them the readings of Z and R in the other three columns. These three values of A are averaged, and the average used in the formula for finding β . Thus four values of β are found which are averaged. This average value of β is the specific threshold of the tissue in question, since it equals the threshold that would be shown had the tissue no resistance. The horizontal columns of R on the card are the values of the tissue resistance (unknown), and are

* MARTIN: *Loc. cit.*, p. 76.

found by means of a Wheatstone bridge against known resistances. The value under R' is the resistance of the secondary coil, which must be added every time to the tissue resistance to give the total resistance of the secondary circuit.

We encountered great difficulty in obtaining subjects. As may readily be seen, constant supervision was advisable. The hourly readings prevented the subject from occupying his time with anything requiring continuous attention, or from going very far from the laboratory. The subjects were all males between the ages of twenty and thirty-five years, and were all in good health.

Experimental procedure. — The subject was shown into the room provided for him, was comfortably seated, and told to dip the first and second fingers of his left hand into the liquid electrodes. He was then instructed to press a telegraph key with his right hand whenever he felt any sensation. All were made to understand that strict attention was essential to the success of the observations. Owing to the building in progress just outside of the windows of the laboratory, we closed windows and door in every case. It is an interesting fact that in the preliminary experiments, for which we had the subjects facing an open window, the thresholds were very irregular due to a distraction of the attention. The preliminary experiments were performed in a room containing a complicated apparatus within the subject's range of vision. To secure uniform attention we changed the subjects from this room to one in which they faced a blank wall. Another precaution was that we took pains to have the subject absolutely comfortable, especially his left arm, which rested on the arm of the chair in such a way that the natural position was with his fingers in the electrodes. We have found that a subject usually does as well the first time he uses the apparatus as when he is more experienced. We always took the precaution, however, in case we had a new subject, of administering several shocks to familiarize him with the sensation, before proceeding to take the readings. The stimulating apparatus is in a different room from that in which the subject sits, nearly fifty feet away and separated from it by two walls. The subject was merely instructed to press the telegraph key, which was within easy reach of his right hand, whenever he felt any sensation. This telegraph key communicated with the operator in the other room.

The electrodes are made of two glass tubes, through the bottom

of which wires from the secondary coil are sealed. The protruding ends of the wires are covered with a layer of clean mercury about 0.5 cm. deep. This is covered to a depth of 10–12 cm. with a strong sodium chloride solution (1 to 15 per cent). The tubes are of ample size and do not compress the fingers. It has been found by experiment that the depth of immersion of the fingers of the subject does not affect the value of β .⁹

After the subject had been comfortably placed, the operator began by “shocking” with the secondary coil 23 cm. from the zero position. Only “break” shocks were used, the “make” shocks being short-circuited by the “make-and-break key.”¹⁰ The coil was then moved in, *i. e.*, towards the zero position, 0.5 cm. at a time until the subject signalled. The coil was then pushed out 0.5 cm., and then pushed in a millimetre at a time until the subject signalled. The coil was then pushed in a few millimetres and then drawn out a millimetre at a time until the subject stopped signalling. The secondary was then drawn out 0.5 cm. further and the operation twice repeated. We found that the threshold was usually 1 or 2 mm. lower when the coil was being pushed away from the zero position than when it was moved towards it. To make the results uniform we accepted the readings only when the coil was being pushed toward the zero position. We also allowed 10–20 seconds to elapse between shocks to preclude the possibility of summation. We then placed a resistance of 10,000 ohms in the secondary circuit and repeated the procedure, and similarly with two higher resistances in the secondary circuit. The primary current was read by a Weston direct-reading ammeter and regulated by a resistance box with a rheocord as a fine adjustment.

As soon as the four thresholds for the four secondary resistances were determined, a switch was thrown whereby the tissue was placed in circuit with a Wheatstone bridge. The resistance of the tissue was then quickly determined by the Kohlrausch method. The entire procedure usually consumed less than ten minutes.

Observed diurnal variations. — Hourly readings of the sensory threshold between 8.30 A. M. and 8.30 P. M. were made upon five different subjects. One of the subjects served for two experiments, so that we have six separate sets of readings, although only four of them

⁹ MARTIN, PORTER, and NICE: *Loc. cit.*

¹⁰ MARTIN: *Loc. cit.*, p. 63.

HOURLY VARIATIONS IN SENSORY THRESHOLD — β UNITS.

Subject.	B.	G.	F.	P.	N.	N.	M.	O.	K.	N.	P.	M.	B.	L.	F.	F.	Hourly mean β .
Hour 8.30 A. M.	88	55	88	110	85
" 9.30 "	83	50	86	47	98	65	72
" 10.30 "	80	48	80	55	66
" 11.30 "	75	60	91	..	98	62	75	..	72	85	122	72	..	81
" 12.30 P. M.	75	73	87	..	95	70	80
" 1.30 "	70	62	86	..	88	79	86	96	106	75	..	83
" 2.30 "	80	52	83	65	86	110	114	100	111	76	88
" 3.30 "	67	70	..	82	110	117	121	127	97	110	102	100
" 4.30 "	89	69	..	115	102	104	..	97	..	102	129	120	103
" 5.30 "	78	70	..	85	80	140	91
" 6.30 "	91	46	..	60	82	112	78
" 7.30 "	79	44	..	76	118	68	77
" 8.30 "	73	40	57
Individual mean }	80	57	86	95	73	90

¹ The results reported in column one are from a twenty-four-hour experiment which began at 4.30 P. M. on one day and ended at 3.30 P. M. the following day.

are complete. Besides these a number of single readings were made upon additional subjects at various hours on different days. Our results are summarized in Table I. The first six columns of this table give the results of our all-day experiments; in the remaining columns are set down the isolated readings. The values given in any particular

vertical column were all obtained from a single subject, although not necessarily on a single day, save in the cases of the all-day experiments.

Significance of the diurnal variation. — To develop the significance of our observations on diurnal variation of the sensory threshold, and to facilitate the comparison of this variation with variations in other phases of bodily activity, we have averaged the results and plotted the averages in two sorts of curves. These curves are presented in Fig. 1. The first of them (the upper continuous line) represents the curve of mean irritability. The mean threshold for each hour was obtained by

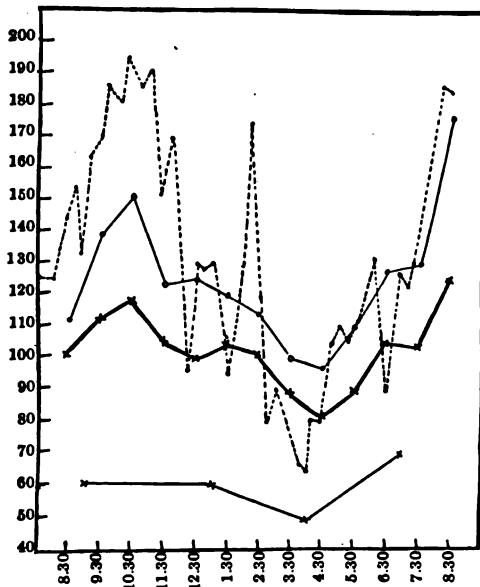


FIGURE 1. — Diurnal variations in nervous activity.

The upper continuous line is the curve of mean irritability. The heavy continuous line is the curve of "relative" irritability, derived by reducing the observed thresholds to a basis of 100. The dotted line is Lombard's curve of ergographic output. The lowest continuous line is drawn from the table of reaction times published by Ostankow and Gran.

averaging together all the thresholds for that hour given in Table I. Since we wished our curve to be one of irritability, and inasmuch as a high threshold signifies low irritability and *vice versa*, we have taken the reciprocal of the threshold as the index of irritability. To make the units of convenient size, the values of reciprocal β were multiplied by 10^4 . From the values thus obtained the curve of mean irritability was plotted.

As a check upon the validity of this curve, and especially to insure

that errors had not entered through the inclusion of observations upon subjects who might, by chance, be super-irritable or sub-irritable, a curve of relative irritability was plotted from the results obtained in the six all-day experiments. The separate observations were reduced to a common basis according to the following scheme:

The mean threshold for each subject was first determined, and then the proportion — mean threshold : 100 = particular observation : X — was solved for each individual threshold. The hourly mean of the "reduced" thresholds was calculated, and the reciprocal of this hourly mean multiplied by 10^4 gave the relative irritability for that hour.

The curve of relative irritability appears as the heavy continuous line of Fig. 1. It corresponds, as the figure shows, very closely with the curve of mean irritability. According to both the curves there is a period of high irritability between ten and eleven in the morning; a fairly continuous decline in irritability till about four-thirty P. M., when irritability is at its lowest; and a steady increase in irritability again which continues till the end of the experiments at eight-thirty in the evening.

A comparison of our curves of irritability with the curves of Lombard¹¹ for ergographic fatigue, and of Ostanikow and Gran¹² for reaction time, brings out some interesting and suggestive relations. To facilitate the comparison, Lombard's curve is reproduced as the dotted line of Fig. 1. The results of Ostanikow and Gran were published in tabular form. We reduced their results to a percentage basis (see above), and then determined the reciprocals of the results, since a short reaction time corresponds to high irritability and *vice versa*. The curve of reaction time is given in the lowest continuous line of the figure. There is a striking parallelism between the curves for irritability and for muscular output, notwithstanding the irritability curves are means of several sets of observations, and the curve of muscular work records but a single series. The time intervals in the observations on reaction time were longer than in our experiments and the number of observations correspondingly smaller. The curve of reaction time does not, therefore, show all the variations seen in the

¹¹ LOMBARD: *Loc. cit.*

¹² OSTANIKOW and GRAN: *Loc. cit.*, quoted from DONALDSON: *Growth of the brain*, London, 1895.

other curves. In general, however, it agrees with them in assigning a higher degree of activity to the morning and early evening than to mid-afternoon.

The obvious conclusion to be drawn from the general parallelism of the curves of Fig. 1 is that the diurnal variation manifest in them is in the main a central rather than a peripheral phenomenon. This conclusion has its bearing in relation to our work, in that it tends to confirm the idea that the sensory threshold is an accurate index to the general nervous state of the subject, — an idea which forms the logical basis for the suggestion that determinations of sensory thresholds may be of value in the study of neurological and psychopathic cases.

SUMMARY.

The irritability of normal adult males, as measured in terms of sensory thresholds for faradic stimulation (Martin method), shows a diurnal variation such that there is a period of high irritability between ten and eleven in the morning; a steadily declining irritability to a minimum at about four-thirty in the afternoon; and a subsequent gain in irritability continuing till the end of our experiments at eight-thirty in the evening.

The diurnal variation in irritability agrees closely with Lombard's observations on ergographic output, and in a general way with the results of Ostanikow and Gran on reaction time.

The conclusion is drawn that diurnal variation is a central rather than a peripheral phenomenon, and that the sensory threshold is, therefore, a reliable index to the general nervous condition of the subject, and may be used as such in studies of abnormal individuals.

THE INFLUENCE OF ALCOHOL UPON REFLEX ACTION IN THE FROG.

BY IDA H. HYDE, RUTH SPRAY, AND IRENE HOWAT.

[From the Physiological Laboratory of the University of Kansas.]

THE following questions form the basis of the present research:
How soon, after administering a minimal and also stronger dose of alcohol, does a change in reflex time appear? How long does the change last? When do the reflex actions cease? For how long a period are they absent? When does the reflex time become normal again?

The experiments were conducted on both spring and normal frogs of the species *Rana esculenta* and *pipiens*, secured from a pond near by. They weighed from 25 to 60 gms. and possessed well-marked pigment spots. During the whole period of experimentation the frogs were kept in moist moss in high glass jars the sides of which were covered with dark paper.

Careful testing of most of the pigment spots showed that they may be regarded as peripheral sensory organs. Those on the head are innervated by branches from the cranial nerves, and those on the trunk and legs by spinal nerves. Some of the spots have a fairly constant reflex time, and others are peculiarly irritable. The same spot also varies more or less in its reaction time at different times of the day, so that each one may be said to have its own reflex time. Those spots were finally selected for study that proved after prolonged testing most reliable and constant in their reactions. The position of these spots is given in Fig. 1.

It was important to find a stimulus that was of a constant definite strength, and that would neither injure nor fatigue the peripheral nerve endings. Pure neutral filter paper, 3 millimetres square and moistened with 8 per cent pure acetic acid, was chosen. The acid paper was placed upon the desired spots, by means of long delicate forceps, with great care to exclude sight and pressure stimuli. That

these errors were avoided was proved by control experiments during which the frog's eyes were carefully covered by a specially devised hood.

By the reflex time is meant the interval between the moment the paper touched the skin and the moment at which the frog made an

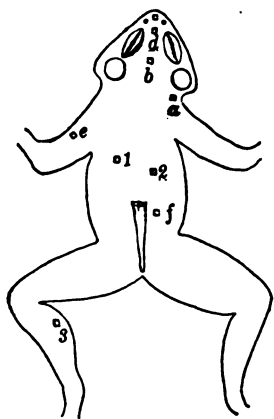


FIGURE 1.—The location of the pigment spots employed in the experiments. *a*, innervated by cutaneous branch of 10th. *b*, innervated by maxillary branch of 5th. *c*, innervated by ophthalmic branch of 5th. *d*, innervated by ophthalmic and superior maxillary. *e*, innervated by branch of 2d spinal. *f*, innervated by branch of 7th spinal. 1 and 2, innervated by branch of spinal nerves. 3, innervated by branch of sciatic.

attempt to remove it. It was found that if the acid was not rubbed off within one minute it never would be. As soon as the attempt was made, or if it was not made in one minute, the spot was washed with fresh water. Thus fatigue and injury to the nerve ending in the spot was prevented, as was proved by testing on the opposite side of the body the corresponding spot that had not been stimulated before. By this means and also from control experiments on unalcoholized frogs with 8 per cent acetic acid, it was shown that fatigue of the peripheral nerve ending due to the stimulus played a subordinate part in the results.

Fixed doses of pure alcohol, varying from 10 to 95 per cent, were tested. The dose determined for the different investigations consisted of a definite number of cubic centimetres of the desired per cent of alcohol per 10-gm. frog.

The alcohol was put into the stomach in gelatine capsules, or through a pipette, or by subcutaneous injections into a lateral lymph sack. The last method proved most satisfactory, because this manner of administration annoyed the frog less, and it was essential to avoid unnecessary excitement or stimulation in order to keep the conditions uniform throughout. The alcohol has its depressing effect about five minutes earlier when given per stomach; it is absorbed more readily than when given subcutaneously.

The method of experimentation required that the reflex time of each one of the sensory pigment spots of a series be first secured from

an unalcoholized frog. The frog that was to be experimented on, moreover, must have shown from repeated tests that the responses were constant for long periods of time. The reflex time for each spot having been secured, the desired dose of the alcohol was given and at once every spot of the series was tested. This procedure was repeated every ten minutes for about two hours of experimentation, and again six or twenty-four hours later, allowing an interval of ten minutes' rest between each test. The turn-over, compensatory, and swimming reflexes were also tested. Table I illustrates the method of recording the observations. While one of us recorded the reaction time, the other applied the acid to the spots and swabbed it off immediately after the frog reacted. If the frog did not react, the acid was allowed to remain one minute and was then washed off.

It was of interest to find the weakest dose of alcohol that produced a change in the reflex time of the cutaneous sensory spots, and whether such a dose had any apparent effect on other reflex actions such as the turn-over, compensatory, or swimming reflex, or upon the general behavior of the frog; also to learn whether increasing doses of alcohol slowed or quickened the reaction time; also the duration of the effect, and whether it varied with varying intervals of time. The doses varied from 0.05 c.c. of 15 per cent per 10-gm. frog up to 0.6 c.c. of 50 per cent, and even fatal doses of 1 c.c. of 95 per cent alcohol per 10-gm. frog were given.

It was demonstrated that each spot had its own reflex time and its own degree of irritability, and that some were more resistant to the influence of alcohol than others. Spot "C" (Fig. 1), for instance, was especially resistant; the acid paper was rubbed off within one second and weak doses of alcohol that affected the reflex time of all the other spots produced no effect upon this spot. Not until a dose of 0.3 c.c. of 30 per cent alcohol per 10-gm. frog was given did the spot fail to respond, and then only for a short time. Larger doses, 0.6 c.c. of 50 per cent alcohol per 10-gm. frog, which suspended the spot reflex and depressed the turn-over and other higher reflexes, would, however, cause a loss of response from this spot as well.

It was shown that quantities of alcohol so small as to produce apparently no effect whatever upon the frog have a decided influence upon the reflex centres of some of the cutaneous areas; at least in so far as to lower their irritability and prolong their reaction time. When once

4.48	43	56	13	1	59	5	6	c
	26	31	3	2	40	..	-	d
	43	25	40	3	48	52	4	e
	32	..	- ⁵	a	60	11	11	1
	10	19	9	b	44	47	2	2
	45	46	1	c	8	13	5	3
	12	..	-	d	57	5	8	a
4.56 ⁴	27	50	23	e	28	35	7	b
5.00	60	5	5	1	50	52	2	c
	38	42	4	2	30	31	1	d
	56	..	-	3	47	48	4	c
	35	..	-	a	34	43	9	1
	36	..	-	b	4	11	7	2
	20	21	1	c	1	15	14	3
	50	..	-	d	13	..	-	a
5.10	26	40	14	e	52	54	4	b
5.20	11	17	6	1	16	17	1	c
	2	15	13	2	42	..	-	d
	5	..	-	3	29	42	13	c
	26	..	-	a

10.25⁷
A. M.

⁴ Injected a dose of 0.1 c.c. per 10 gm. of 30 per cent alcohol.
⁵ (-) means failure to react during one minute, after which the spot was immediately washed.
⁶ Turn-over, swimming, compensatory, and equilibrium reflexes normal.
⁷ Mar. 29.

¹ Frog, 41.6 gm., Mar. 28.
² Stimulus, 0.8 per cent acetic. Time in seconds. From normal frog.
³ Turn-over, swimming, compensatory, and equilibrium reflexes normal.

affected by alcohol, these centres do not become normal in their response for some time.

From repeated tests in a large series of experiments it was seen that doses less than 0.05 c.c. of 15 per cent alcohol per 10-gm. frog had no more effect upon the reflex time than had an equal amount of Ringer solution. But beginning with 0.1 c.c. of 15 per cent alcohol, all the other doses that were given had a depressing effect upon the reflex actions; that is, they caused the interval between stimulation and response to be prolonged or the reflex action to be lost or become irregular in its response, within ten minutes. This change lasted from one to a half hour after injecting the alcohol. Often 0.1 c.c. of 15 per cent alcohol and 0.1 c.c. of 30 per cent alcohol produced no difference in the same frogs. The effect of the dose, it seems, depends largely upon the condition of the frog and the susceptibility of the special sensory spot tested. With doses from 0.3 c.c. of 15 per cent to about 0.3 c.c. of 30 per cent alcohol the turn-over, compensatory, and equilibrium reflexes are not depressed, but the frog seems very irritable and restless, often failing to remove the stimulus in its attempts to jump out of the jar. With doses of 0.3 c.c. of 30 per cent alcohol, the frog immediately gets sluggish and a greater depression of the reflex time is produced, not only in the skin reactions but also in the higher reflexes; even spot "C" sometimes fails to react. Doses of 0.5 c.c. of 30 per cent alcohol cause a loss of all skin reflexes and of muscle tone. Following such a dose the frog frequently lies flat and is sluggish, has labored respiration, and the turn-over, equilibrium, and other higher reflexes are lost for five minutes or more. Later, in some cases, the frog moves about restlessly and makes only weak attempts to remove the stimulus; or the frog may seem apparently lifeless and all skin reflexes may be abolished for about one hour. With larger doses 0.6 c.c. of 50 per cent alcohol, the turn-over and higher reflexes are lost within ten minutes and for one to two hours. But in twenty-four hours they are again normal, not, however, the skin reflexes. Doses of 0.8 c.c. of 95 per cent alcohol proved toxic.

It was observed that the spots fail to respond immediately if at all after a given dose and the depression increases and is of longer duration with increase in dose until with a dose of 0.6 c.c. of 50 per cent alcohol, the skin and higher reflexes are all lost for from one to two hours, and the skin reactions are not normal again even

after twenty-four hours. Sometimes following such a dose respiration ceases and tetanic convulsions appear, lasting for about an hour.

In connection with these experiments it was of interest to determine what some of the doses of alcohol per gram animal would amount to for man. Sherry, orange, and port wine contain from 15 to 20 per cent alcohol, claret and white wine from 10 to 14 per cent, beer from 4 to 10 per cent, with a large amount of other ingredients, while whiskey and brandy contain from 40 to 50 per cent.

A dose of 0.1 c.c. of 15 per cent alcohol per 10-gm. frog, which would produce no perceptible change in the behavior of the frog but would cause an immediate depression, and in some instances a loss of reflex action lasting for an hour or more, and not giving place to normal after six hours, would be equivalent to one pint of sherry or orange or port wine or 1.13 pints of claret or two pints of strong beer, for a man of average weight, or about 64.7 kilos.

In general we may say that alcohol in small quantities, even when no perceptible change in the behavior of the frog is noticed, lowers the reaction time. When the reflex centres have once been affected, even slightly, they do not become normal again for six to twenty-four hours. Therefore even small quantities of alcohol exert a depressing chemical action upon certain parts of the nervous system; the muscle tone, and vasomotor, and cutaneous thermal reflex actions may be indirectly affected. The larger the dose the greater the effect and the quicker it comes on after giving the alcohol. The effect of the alcohol depends somewhat upon the individual. Some frogs became excited and others sluggish, but there was always a depression of the nervous reflex centres. Certain cutaneous nerve organs in the frog are more irritable and also more susceptible to the influence of alcohol than others. The skin reflexes are affected by smaller quantities of alcohol than are the turn-over, swimming, compensatory, and equilibrium reflexes, and they become normal again more slowly. Alcohol taken in sufficient quantities, 0.6 c.c. of 50 per cent for instance, causes loss of all reflexes, a loss of muscle tone, unconsciousness, and convulsions similar to those caused by strychnine poisoning.

SUMMARY.

Certain sensory spots in the frog's skin differ not only in irritability and reflex action, but in susceptibility to the influence of alcohol, and they vary more or less in their reflex time at different intervals of the day.

The sensory spots or cutaneous sensory organs that were experimented on are affected by much smaller quantities of alcohol than are the turn-over, swimming, compensatory, or equilibrium reflexes. When the dose is sufficient to produce any effect at all, it is always a depressed or slowed response to the stimulus, never a stimulating effect or a shorter reflex time.

A dose less than 0.05 c.c. of 15 per cent alcohol per 10-gm. frog produces no more change in the reflex time of some of the cutaneous sensory organs than does the same dose of Ringer solution. But a dose of 0.3 c.c. of 30 per cent alcohol, though seeming to stimulate by causing the frog to become irritable and active, nevertheless produces a depression, or causes irregular responses, or an entire loss of the reflex action. These changes were observed in all of the spots experimented on excepting (*c*), which was not influenced by weak doses.

Moreover, these changes appear within ten minutes after injecting the alcohol, and persist for from one to one and a half hours. Doses above 0.3 c.c. of 30 per cent to 0.6 c.c. of 50 per cent produce within five minutes great changes in the frog's behavior and appearance. Often the frog assumes a flat, sluggish, dazed attitude. Both the cutaneous and higher reflexes are lost. The muscle tone is much decreased, respiratory activity is not perceptible, and tetanic convulsions may set in with the stronger doses. This condition may continue from one half to two hours, but the reflex time of the cutaneous sensory organs does not become normal again even after twenty-four hours.

Alcohol in quantities too small to produce any apparent effect upon the frog has nevertheless a decided influence upon the reflex centres of some of the cutaneous sense organs, at least in so far as to lower their irritability and reaction time. When these centres are once affected by alcohol, their reflex responses do not again become normal for from six to twenty-four hours.

When the sensory spots fail to respond to a dose, a larger dose has

Influence of Alcohol upon Reflex Action in the Frog. 31

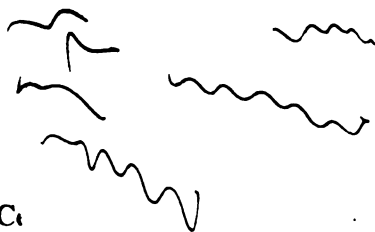
an immediate effect. The depression increases, and is of longer duration as the dose increases, until with 0.6 c.c. of 50 per cent alcohol reflexes from the sensory spots and the higher reflexes as well, are either much retarded or lost entirely for several hours. One cubic centim or of 95 per cent alcohol is toxic.

It was observed that even small quantities exert a depressing chemical action upon certain parts of the nervous system, affecting the muscle tone and the vasomotor and cutaneous thermal reflex actions to a degree depending upon the condition of the nervous centres.

Doses of 0.1 c.c. of 15 per cent alcohol per 10-gm. frog would be equivalent to one pint of sherry or orange or port wine or 1.13 pints of claret or two pints of strong beer for a man of average weight. This dose caused in the frog a depression of the reflex time of the cutaneous sensory organs.

P. J. ...
3. ...

3.



C₁
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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH. — IV. THE INFLUENCE OF THE CONTRACTIONS OF THE EMPTY STOMACH IN MAN ON THE VASOMOTOR CENTRE, ON THE RATE OF THE HEART BEAT, AND ON THE REFLEX EXCITABILITY OF THE SPINAL CORD.

By A. J. CARLSON.

[From the Hull Physiological Laboratory of the University of Chicago.]

THE work reported in this paper was planned and partly completed before definite evidence was secured on the nature of the causal relationship between the contractions of the empty stomach and the sensation of hunger. It would seem that if the processes of the central nervous system known as states of hunger give rise to the contractions of the empty stomach, the state of hunger would likely be associated with increased reflex excitability and possibly with an increased motor innervation of sympathetic neuromuscular mechanisms besides those of the alimentary tract. The knee jerk and the tonus of the urinary bladder were selected as points of attack. There are some data in the literature on the influence of hunger on the knee jerk, but in those investigations no account was taken of the periodicity of the hunger states. No observations have therefore been made on the condition of the knee jerk during the individual hunger pangs and the corresponding contractions of the empty stomach. This can readily be done in our man V. It was realized, of course, that positive results would not be entirely conclusive; the possibility would remain that the changes in reflex excitability and in motor innervation are secondary effects of the states of hunger initiated by the stomach contractions.

1. *The rate of the heart beat.* — The rate of the heart beat was recorded by means of an Erlanger sphygmomanometer, the cuff being fixed to the right arm and the writing point adjusted to the kymograph used for recording the stomach contractions. In two of the experi-

ments V. was lying down; in the rest of the series he was sitting or rather half reclining in a Morris chair.

The pulse of V. is of normal rate and regularity. When the empty stomach is relatively quiescent and the man is sitting quietly or

TABLE I.

SUMMARY OF OBSERVATIONS ON THE RATE OF THE HEART BEAT IN V. DURING THE PERIODS OF STRONG CONTRACTIONS OF THE EMPTY STOMACH (HUNGER CONTRACTIONS).

Number of experiment.	Number of stomach contractions and pauses counted.	Rate of the pulse.			
		Before the contraction period.	During the individual contractions.	During pause between the contractions.	Five minutes after cessation of the contr. period.
I	8	76	81	79	77
II	18	78	87	82	80
III	11	75	81	78	76
IV	13	76	83	81	75
V	6	72	81	79	74
VI	28	70	85	82	74
VII	12	68	78	74	70
VIII	11	73	79	76	74
IX	4	..	80	74	..

lying down, the rate of the pulse on different days was found to vary between 68 and 75 per minute. During the period of strong stomach contractions the rate of the heart beat is increased. At the cessation of the contraction period the heart rate becomes slower again. The return to the normal rate is gradual. If the motor activities of the empty stomach are very vigorous, so that the pauses between the contraction periods are relatively short, the pulse rate may not return quite to the normal during these pauses. The average increase in the rate of the heart beat during the hunger contractions of the stomach is eight to ten per minute (see Table I). But strong individual contractions or tetanus periods may show an increase of thirty beats per minute.

When the details of the contraction periods are further analyzed, it is found that the greatest acceleration of the heart beat is on the whole synchronous with the individual strong contractions, and that the pauses between the contractions usually show less acceleration. Occasionally the relation is the reverse. This relation may be illustrated by giving the detail of the final half of one contraction period.

Condition of the stomach.	Rate of the pulse.	Condition of the stomach.	Rate of the pulse.
Contraction	84	Quiescence	84
Quiescence	82	Contraction	90
Contraction	88	Quiescence	84
Quiescence	85	Contraction	90
Contraction	85	Quiescence	89
Quiescence	82	Contraction	91
Contraction	85	Quiescence	82
Quiescence	84	Contraction	90
Contraction	84	Quiescence	83
Quiescence	84	Contraction (tetanus)	92
Contraction	88	Quiescence (following the tetanus)	80

While the rate of the heart beat is usually less during the pauses than during the contractions, the rate during the pauses is always greater than during the much longer periods of quiescence between successive periods of the hunger contractions. On the whole, acceleration of the heart beat is directly proportional to the amplitude of the hunger contractions and is therefore greatest during the hunger tetanus.

2. *Vasomotor changes synchronous with the contractions of the empty stomach.* — The left hand and forearm of V. were inclosed in a plethysmograph connected with a water manometer for recording the volume. The plethysmograph and rubber tube connection with the manometer were filled with water at 37° C. The hand and arm were protected from the water by a delicate rubber glove or by a thick coating of vaseline. During the experiments V. was either lying down on a couch or reclining in a Morris chair. After being adjusted in a perfectly comfortable position V. was instructed not to move unless getting my permission. He was also instructed to notify me as soon as he felt any discomfort because of his position.

It is well known that the vasomotor centre is acted upon not only

by practically all afferent impulses, but also by the conscious processes in and by the centrifugal impulses from the cerebrum. None of these disturbing factors can be controlled completely. The most we can do is to endeavor to make the external conditions and the cerebral processes as uniform as possible. This was attempted in two ways during the experiments. V. was permitted to read stories; he was required to add figures; or his eyes were covered and he was instructed to think

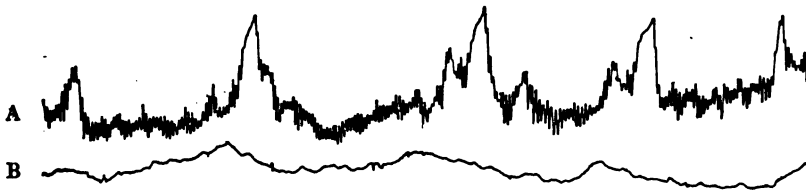


FIGURE 1. — About one third the original size. A, contractions of the empty stomach; B, plethysmograph record of volume of the left arm. Showing a vasomotor rhythm parallel with the strong hunger contractions of the empty stomach.

of nothing in particular. Aside from the varying cerebral states, auditory stimuli were the greatest disturbing factors. The university campus is probably as quiet as can be expected in a large city, but the whistle of locomotives, steamers, and factories, the gong of the street cars, and the "honk" of the automobiles reach the tympanic membranes with great force, especially when one endeavors to exclude all sensory stimuli as far as possible.

The periods of strong contractions of the empty stomach are synchronous with great variations in the vasomotor tone, and in most cases the vasomotor variations exhibit a rhythm similar to that of the stomach. The reader's attention is called to two types of this parallelism illustrated by the tracings reproduced in Figs. 1 and 2. There is an increase in volume of the arm (vaso-dilation) *pari passu* with an increasing tonus of the stomach and with the beginning of the individual contractions, but the arm begins to shrink (vaso-constriction) before the stomach contraction has reached its maximum (Fig. 1). Or the volume of the arm shows a definite increase parallel with the strong contractions, and a corresponding diminution in the arm volume during the stomach pauses (Fig. 2). These two types of vasomotor rhythms were the ones usually obtained during periods of moderate hunger contractions, when V. was sitting or lying down comfortable and with no indications of restlessness. If the

experiments were continued for long periods (four to six hours), so that the hunger contractions and the hunger tetanus became very strong, the vasomotor variations were in evidence, but the rhythms were rarely synchronous with the stomach rhythms. In such instances V. gave unmistakable signs of restlessness. Under these conditions the vasomotor rhythm may be slightly faster or slightly

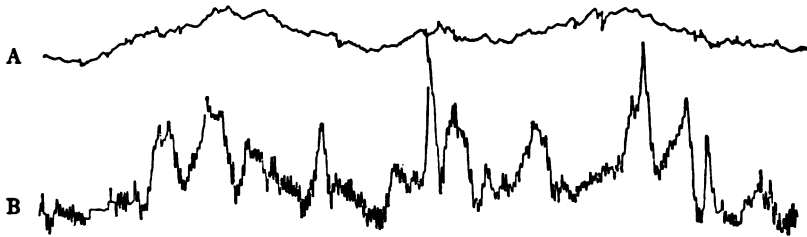


FIGURE 2. — About one third the original size. *A*, contractions of the empty stomach; *B*, plethysmograph record of the volume of the left arm. Showing vaso-dilation parallel with the strong hunger contractions.

slower than the stomach rhythm, or the two rhythms may be practically identical, but the contraction phase of the stomach activity may be synchronous with a decrease in the volume of the arm (vaso-constriction).

Before the appearance of the first period of hunger contractions after a meal, and during the pause or relative quiescence of the stomach between the periods of moderate hunger contractions, another type of vasomotor rhythm appears on the plethysmographic records. A tracing showing this rhythm is reproduced in Fig. 3. Considerable attention was given to this rhythm, because the rate of it suggested some correlation with the "twenty-seconds rhythm" of the empty stomach. These slight variations in the arm volume are frequently irregular, so that its rate cannot be made out with a certainty, but when the fluctuations are fairly regular the rate corresponds closely to the "twenty-seconds rhythm" of the empty stomach (Fig. 3). The contraction and relaxation phases of the two rhythms do not seem to correspond, but that is of little significance in view of probable difference in the latent time of the respective neuromuscular apparatus as well as of the recording devices. This type of vasomotor rhythm is most marked when the "twenty-seconds rhythm"

of the stomach is the strongest, that is, at the beginning of a period of strong hunger contractions.

In view of the fact that the vasomotor centre is acted on by so many factors, central and peripheral, the parallelism between the vasomotor tone and the motor activity of the empty stomach during the hunger period is too regular to be accidental. When the synchrony

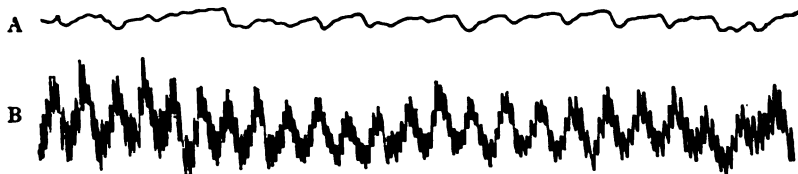


FIGURE 3. — Two thirds the original size. *A*, plethysmograph record of the volume of the left arm; *B*, contractions of the empty stomach ("twenty-seconds rhythm"). Showing a vasomotor rhythm almost parallel with the "twenty-seconds rhythm" of the stomach.

fails, this is probably due to central or peripheral factors that cannot be controlled or recorded. The question then remains how this synchrony is brought about, and what is the biological significance of it. (1) The co-ordination may be due to associations between the vasomotor centre and the centre for gastric tonus in the medulla; (2) it may be due to a direct action on the vasomotor centre by afferent impulses from the stomach initiated by the stomach contractions; (3) or, it may be due to the influence on the vasomotor centre of the conscious processes of hunger, which are caused by the stomach contractions. Brodie and Russel¹ and Miller² have studied the effect on the blood pressure of stimulation of the central end of the gastric branches of the vagi in animals under general anæsthesia. The changes in the blood pressure are variable and complicated by respiratory changes and vomiting movements. In dogs and cats the primary effects may be either increase or decrease in the arterial pressure, while in rabbits the stimulation seems to cause a rise in the blood pressure only. It will probably be difficult to secure experimentally the selective stimulation of the afferent gastric nerve fibres that are stimulated by the contraction of the empty stomach. There may be some connection between the vasomotor rhythms described above

¹ BRODIE and RUSSEL: *Journal of physiology*, 1900, xxvi, p. 92.

² MILLER: *Archiv für die gesammte Physiologie*, 1911, cxliii, p. 21.

and the well-known Traube-Hering blood pressure variations, as the latter are frequently induced by experimental interferences with the vagi.

The vasomotor disturbance synchronous with the periods of strong hunger contractions of the empty stomach is probably an important factor in the feeling of weakness and faintness so frequently associated with hunger in man. These conditions are not primarily due to decrease in the available food materials in the blood, because they disappear after eating before any considerable portion of the ingested food has been absorbed.

Faintness, headache, weakness, etc., as concomitants of hunger states, vary with the individual, and it may be questioned whether any of them constitute a necessary part of the hunger complex. The writer has certainly experienced intense hunger not associated with any of the above symptoms. When considered biologically, it seems that the pseudo weakness of hunger, and the disturbance of the vasomotor mechanisms would tend to defeat the very purpose of hunger, the securing of food. The essential relation of the hunger processes to the vasomotor mechanism must therefore be worked out primarily on the lower animals.

3. *The relation of the contractions of the empty stomach to the reflex excitability of the spinal cord.* — The results of the more recent studies on the knee jerk or patellar reflex seem to support the view that it is a true reflex action. But even if it should finally prove to be not a reflex, but a direct muscular response, it can nevertheless be used as an index of the reflex excitability of the spinal cord, because the myotatic response of the muscle is contingent upon and varies directly with the tonus reflex.

In this series of experiments Mr. V. was reclining comfortably in a Morris chair. The left thigh down to the knee was fixed rigidly in a plaster cast so that the pendent leg swung free. The stimulus consisted of a steel hammer weighing 350 gm. falling through an arc of 20° .³ The device for measuring the degree of extension of the leg was adjusted to record on the same drum and perpendicularly to the manometer record of the stomach contractions. Mr. V. was not told of the nature or aim of the experiments, so as to avoid conscious augmenta-

³ I am under obligation to my assistant, Mr. H. O. LUSKY, for construction of most of the apparatus for this phase of the work.

tion or inhibition. A screen shut out the view of the left knee and the stimulating hammer, so as to avoid the errors from anticipation. The stimulating hammer struck the tendon through a single layer of thin cloth. The pivot of the stimulating hammer was fixed, the chair with the plaster cast was also rigidly fixed so that the impact of

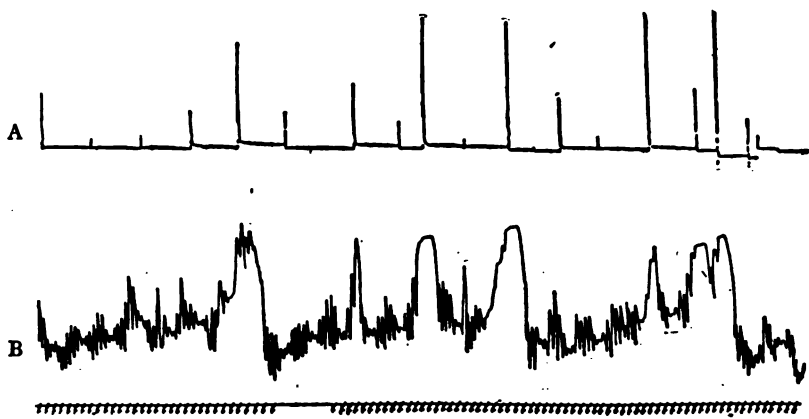


FIGURE 4. — About one half the original size. *A*, record of the knee reflex; *B*, contractions of the empty stomach. Showing augmentation of the knee reflex during the hunger contractions. Time, five seconds.

the hammer was made at the same point of the tendon, not only throughout each single series of experiments, but also from day to day.

The knee reflex of V. is relatively sluggish, requiring a considerable strength of blow to secure maximum response. After some trials I finally fixed on a minimal strength of the stimulus, and this was used in all the work here reported. This stimulus was given by the fall of the hammer through an arc of 20° . When V. was sitting quiet, external stimuli excluded as far as possible, and the empty stomach in relative rest, this stimulus produced no effect in about 50 per cent of the tests, while in the other 50 per cent of the tests a slight contraction followed. These contractions were never maximal under the above condition of V. This minimum stimulus was chosen because I found it easier to keep V.'s attention from the knee and hammer when the impact was slight, and after it had become evident that hunger contractions of the stomach augment the knee jerk. A minimum stimulus cannot, of course, be used to investigate conditions that depress the knee jerk.

The records of this series show without exception that there is a

marked increase in the reflex excitability of the spinal cord simultaneously with the strong hunger contractions of the empty stomach. The reflex excitability usually falls to normal level during the pauses between the single contraction, and after the strong hunger period it appears to be somewhat lower than normal. The hunger contractions

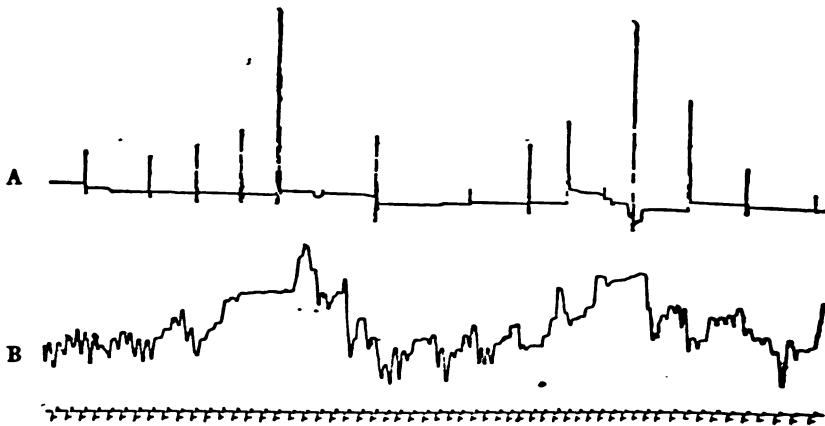


FIGURE 5. — About one half the original size. *A*, record of the knee reflex; *B*, contractions of the empty stomach. Showing a gradual increase in the augmentation of the knee reflex parallel with the shortening phase of the single contractions of the empty stomach. Time, five seconds.

of the empty stomach frequently increased the reflex response of the cord to such an extent that the standard minimal stimulus caused a maximal response. The degree of augmentation of the reflex is, on the whole, proportional to the amplitude of the stomach contractions, and this can be shown frequently during the shortening phase of a single contraction (Fig. 5). There are many exceptions to this last statement, to be sure. But this is to be expected in view of the fact that under conditions as nearly uniform as possible two successive stimuli of equal character and intensity rarely give two successive responses of equal magnitude.⁴

Some attention was paid to the question whether this augmentation of the reflex excitability of the central nervous system synchronous with the hunger contractions of the stomach is in evidence before the contractions have given rise to the conscious hunger pangs. This much is certain, that the augmentation is greatest at the height of the

⁴ DODGE: *Zeitschrift für allgemeine Physiologie*, 1910, xii, p. 21.

stomach contraction, when the hunger pang is the most intense. The present data do not warrant any statement on the question whether or not the beginning of the augmentation precedes the conscious hunger pang to the same extent that the stomach contraction precedes the hunger pang, because little importance can be attached to slight variations in the amplitude of the reflex response, except when the general average is made significant by the great number of the individual experiments. The stomach contraction is the primary factor or stimulus, whether or not conscious cerebral processes constitute a necessary link in the chain of events that results in augmentation of the reflexes.

Lombard⁵ concluded from experiments on himself that hunger depresses the knee jerk. No account is given of the degree of hunger experienced by L. before lunch and dinner. Possibly L. experienced only appetite and feeble and indefinite "hunger" that may be present in the absence of the strong stomach contractions. In such conditions there is no augmentation of the knee jerk in my subject. It should also be noted that in some cases Lombard found his knee reflex greater before the meal (hunger?) than after the meal.

It may be questioned whether a comparison of the amplitude of the knee reflex before and after a meal is an adequate criterion of the effect of hunger states on the reflex excitability of the spinal cord. It seems to me that this is a comparison between hunger (or appetite) and satiety, and not between hunger and the absence of hunger. The partaking of food when hungry involves many changes of a positive character besides the abolition of hunger. Hence it is clear that there is no contradiction between the present results on V. and Lombard's results on himself. The two series cannot be compared, because the conditions of the subjects were not comparable. I have made no tests on V. before and after a meal, similar to those of Lombard. But when the comparison is made between the state of hunger (as differentiated from appetite) and the absence of hunger, the evidence is conclusive that hunger leads to or is associated with an increased excitability of the cerebro-spinal axis. This condition probably accounts for the irritability, restlessness, and inability to maintain a fixed attention frequently noted in strong or even in moderate hunger.

⁵ LOMBARD: American journal of psychology, 1887, i.

THE RATE OF THE DESTRUCTION OF PTYALIN BY THE PASSAGE OF THE DIRECT ELECTRIC CURRENT.

By W. E. BURGE.

[From the Physiological Laboratory of the Johns Hopkins University.]

THE present investigation was begun to determine what effect, if any, the passage of a direct electric current would have on ptyalin. It was soon observed that, just as the concentration of a solution of silver nitrate is decreased by the passage of the electric current, so the activity of the ptyalin was decreased. This decrease was gradual until complete destruction occurred. It has recently been shown that the passage of a direct current through solutions of commercial preparations of rennin,¹ which contain pepsin as well as rennin, results in the destruction of the pepsin while the rennetic activity of the solutions apparently is unaltered, so that the destruction of the ptyalin was not entirely unexpected. The fact that the enzyme was destroyed by the passage of the current having been established, the gradual decrease in diastatic power that had been noted naturally led to the question whether any quantitative relation exists between the amount of current passed and the amount of ptyalin destroyed.

In the effort to find an answer to this question the methods described below were finally adopted.

Human saliva, diluted one to three with distilled water, was used throughout the experiments, and the electrolysis of this diluted saliva carried out in a glass cylinder, a longitudinal section of which may be seen in Fig. 1.

The diastatic power of the ptyalin was determined by the amount of sugar formed from starch paste. The paste was made by adding 20 gm. of cornstarch to 500 c.c. of distilled water. The beaker containing the mixture was placed in a water bath and cooked for two hours. Ten cubic centimetres of the paste were poured into a long test tube,

¹ BURGE: this Journal, 1912, xxix, pp. 330 *et seq.*

which was then placed in a water bath where it was allowed to remain until the temperature reached 38°C . Two cubic centimetres of the diluted saliva were poured into the paste, and the tube replaced in the water bath for five minutes. At the end of this time the tube was run through a free flame and the contents brought to boiling in thirty seconds, thus stopping the action of the enzyme. The amount of sugar in 10 c.c. of this solution was determined by Pavy's method.

Twelve series of electrolyses were made. Four of these, with duplicates, are reported in this paper.

The experiments were carried out in the following manner: 5 c.c. of human saliva, diluted one to three, were placed in the electrolyzing cylinder. The cylinder was placed in a shaking machine across the electrodes of an electric circuit. The object of the shaking machine, which was run at the rate of four hundred single shakes per minute, was to prevent the accumulation of the electrolytic products at the poles. These products would, of course, have destroyed the ptyalin.

Thirty-five milliamperes of current were passed through the solutions while they were being shaken at the rate named above. The temperature of the electrolyzed solutions never rose higher than 35°C ., and this temperature was reached only in those solutions where the current had passed for twenty minutes. The diastatic power of the electrolyzed saliva was determined, as in the controls, by the amount of sugar formed by 2 c.c. from 10 c.c. of the standard starch paste in five minutes at 38°C .

The results of these determinations may be seen in the table on page 330.

In the column headed by N will be found the number of milligrams of sugar produced in five minutes by 2 c.c. of the diluted non-electrolyzed saliva. In the columns headed by five, ten, fifteen, and twenty minutes respectively will be found figures indicating the amount of sugar

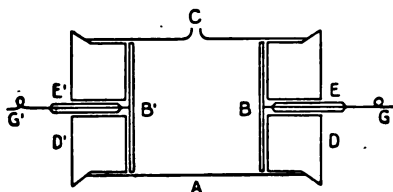


FIGURE 1. — Electrolyzing cylinder (longitudinal section). *A*, cylinder; *B* and *B'*, platinum electrodes; *C*, opening into cylinder; *D* and *D'*, rubber stoppers; *E* and *E'*, glass tubes; *G* and *G'*, platinum wires. The electrolyzing cylinder consists of a glass cylinder 5 cm. in length and 3 cm. in diameter. In each end is a rubber stopper with a central bore through which passes a glass tube. Fused into this tube is a platinum wire connecting with the platinum electrode. The platinum electrodes are discs 3 cm. in diameter and are placed 3 cm. apart. Through the opening liquid may be introduced into or removed from the cylinder.

formed by the diluted saliva that had been electrolyzed for these periods. It will be understood that for each experiment the electrolyzing tube was newly charged with 5 c.c. of the diluted saliva. If we examine Experiment I we find that in 10 c.c. of starch paste 2 c.c. of diluted saliva produced 13 mg. of sugar. We shall see also that after

TABLE I.

		N	5 min.	10 min.	15 min.	20 min.	A	5 min.
		mg.	mg.	mg.	mg.	mg.	mil.-amp.	
After standing six days . .	Exp. I	13.0	7.8	5.8	3.2	+ Feh.	35	10.5Q
	Dupl. 1	10.4	6.2	3.5	+ Feh.	+ Feh.	35	10.5Q
After standing five days . .	Exp. II	13.4	8.9	6.8	3.4	+ Feh.	35	10.5Q
	Dupl. 2	16.6	10.4	4.9	+ Feh.	+ Feh.	35	10.5Q
After standing four days . .	Exp. III	10.0	7.2	4.8	2.5	+ Feh.	35	10.5Q
	Dupl. 3	10.1	8.0	4.0	+ Feh.	+ Feh.	35	10.5Q
After standing two days . .	Exp. IV	10.8	8.6	5.7	2.9	+ Feh.	35	10.5Q
	Dupl. 4	10.8	8.0	5.9	+ Feh.	+ Feh.	35	10.5Q

a similar solution had been electrolyzed for five minutes 2 c.c. produced 7.8 mg.; for ten minutes, 5.8 mg.; for fifteen minutes, 3.2 mg., and that after twenty minutes the diastatic power was practically destroyed. In this last instance only a slight test for sugar was obtained with Fehling's solution.

In the column headed by A the figures show the milliamperes of current passed. The number was constant, thirty-five, throughout the experiments.

In the columns bracketed under Q are found numbers indicating the number of coulombs used in the five-minute, ten-minute, and fifteen-minute periods -- 10.5, 21, and 31.5 coulombs respectively.

In the columns bracketed under X are figures indicating the decrease in the number of milligrams of sugar produced by the electrolyzed saliva.

In the columns bracketed under Y are figures indicating the per-

centage decrease in the amount of sugar formed by the electrolyzed saliva.

In the columns bracketed under Z are figures indicating the percentage decrease per coulomb in the amount of sugar produced by the electrolyzed saliva.

TABLE I.

Q		X			Y			Z		
10 min.	15 min.	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
21Q	31.5Q	5.2	7.2	9.8	per cent 40	per cent 55	per cent 75	per cent 3.8	per cent 2.6	per cent 2.4
21Q	31.5Q
21Q	31.5Q	4.5	6.6	10.0	34	49	75	3.2	2.3	2.4
21Q	31.5Q
21Q	31.5Q	2.8	5.2	7.5	28	52	75	2.7	2.5	2.4
21Q	31.5Q
21Q	31.5Q	2.2	5.1	7.9	20	47	73	2.0—	2.2	2.3
21Q	31.5Q
					30+	50	74+	2.9	2.4	2.4—

Upon examination of Experiment I it may be seen that while 2 c.c. of non-electrolyzed saliva produced 13 mg. of sugar a similar amount after electrolysis for five minutes produced 7.8 mg.; that is, the passage of the current for five minutes reduced the diastatic power of the enzyme by 5.2 mg. of sugar, or to 40 per cent of the amount produced by the non-electrolyzed. The passage of the current for ten minutes reduced it 7.2 mg., 55 per cent of the original activity; for fifteen minutes, 9.2 mg., 75 per cent of the original activity. After the current had passed for twenty minutes, the amount of sugar was too small to determine quantitatively. We may see also that

In five minutes, 10.5 coulombs passed with a reduction of 40 per cent of the activity.

In ten minutes, 21 coulombs passed with a reduction of 55 per cent of the activity.

In fifteen minutes 31.5 coulombs passed with a reduction of 75 per cent of the activity.

In twenty minutes 42 coulombs passed with a reduction of practically 100 per cent of the activity.

If in five minutes there was a reduction in diastatic power of 40 per cent while 10.5 coulombs of electricity passed, it is evident that the rate of destruction was 3.8 per cent per coulomb. Similarly for the ten-minute period where 21 coulombs passed, we find a rate of 2.6 per cent per coulomb, and for the fifteen-minute period where 31.5 coulombs passed, a rate of 2.4 per cent per coulomb. It can be seen from Table I that the average rate of destruction of the diastatic power, as measured by the decrease in this amount of sugar formed, is 2.5 per cent per coulomb, and that this rate is practically the same for all the periods of time (five minutes, ten minutes, fifteen minutes) during which the current was passed.

It might be assumed that the electrolytic products were the cause of the destruction of the ptyalin. To rule out this explanation a part of the electrolyzed saliva from each experiment was kept for varying lengths of time and again tested, as before, for its diastatic power. If the electrolytic products were the cause of the destruction of the enzyme, this destruction should have continued after the current had ceased to pass. Not only should the destruction have continued, but it also should have been greatest in the solutions electrolyzed for the longest time.

The parts of the solutions from Experiment I kept for six days and then tested for their ability to produce sugar are designated Duplicate 1. Similarly, the solutions from Experiments II, III, and IV are designated Duplicates 2, 3, and 4, and were kept for the times indicated in the table before the second tests were made. It was found that the activity of the non-electrolyzed solutions had, in general, decreased, as had also that of the electrolyzed solutions in most cases, and that this decrease in each was to about the same extent. This relation in activity of the electrolyzed solutions may be seen by the comparison of the experiments with their duplicates.

In the case of silver we know that the concentration of the solution of a silver salt is decreased by the passage of an electric current and that the element is deposited on the cathode. In the case of ptyalin

we know only that the activity of the ptyalin is decreased but not what becomes of the ptyalin. The electrochemical equivalent of silver is obtained by weighing the amount of silver deposited on the cathode and dividing by the number of coulombs of electricity passed. An other way of determining the electrochemical equivalent, at least theoretically, would be to determine the decrease in the concentration of the silver solution, which decrease would be equal to the amount of silver deposited on the cathode. We are unable to determine the substance or substances liberated at the poles or destroyed in some unknown way by the passage of the current through ptyalin, but we may determine the decrease in the diastatic potency of the ptyalin, just as we might determine the decrease in the concentration of a silver solution. The gradual destruction of ptyalin by the passage of a direct electric current suggests that a certain homology may exist between the electrochemical equivalent of metals and the rate of the destruction of this enzyme.

CONCLUSIONS.

1. Ptyalin is destroyed by the passage of the direct electric current.
2. This destruction is not due to the electrolytic products.
3. The rate of destruction is uniform and is at the rate of 2.5 per cent per coulomb for the solutions used in this investigation.

A CRITICISM OF THE INDICATOR METHOD OF DETERMINING CELL PERMEABILITY FOR ALKALIES.

By E. NEWTON HARVEY.

[From the Physiological Laboratory, Biology Department, Princeton University.]

EXPERIMENTS on the permeability of various types of cells have shown that the cell membrane (plasma membrane) is very readily permeable to weakly dissociated alkalies but without exception remarkably resistant to the penetration of strongly dissociated alkalies.¹ To the former class belong ammonia and the primary, secondary, and tertiary aliphatic amines; to the latter the inorganic hydroxides and tetraethylammonium hydroxide.

In the experiments use has been made of the indicator method. The cells were stained in neutral red, a basic dye, red in acid or neutral solution (as salt, RCl) and yellow in alkaline solution (as base, ROH). In some cases the dye is held in solution in the cell sap (*Elo-dea*); in others it is precipitated as a tannin-neutral red compound (*Spirogyra*), while in most cases it unites with granules already present in the cell (*Paramoecium* and the cells of marine animals). The penetration time of the alkali may therefore be determined by the color change from red to yellow of the neutral red within the cell.

It must be borne in mind that the indicator is always contained in the cell in some kind of chemical combination, adsorption or absorption, and that there are also present proteins and other substances which may influence the color change, so that it may not be a true measure of the rate of penetration of the alkali. For this reason criticism has recently been directed against the indicator method.² I propose to consider the objections in the following pages. Sodium

¹ HARVEY, E. N. *Journal of experimental zoölogy*, 1911, x, p. 507, and Carnegie Institute of Washington, Tortugas Publications, 1913.

² MCCLENDON, J. F.: *Journal of biological chemistry*, 1912, x, p. 459.

hydroxide has been selected as a type of the strong alkalies and ammonia as a type of the weak alkalies.

This point is of especial interest in view of the following observations which I have repeatedly made and the conclusions I have drawn from them. The word "decolorized" is used to indicate the change in color from red to yellow.

1. Paramœcia are decolorized almost instantly in $n/1000$ NH_4OH , before ciliary movement is affected or the organisms change appreciably in shape. In $n/500$ NaOH decolorization takes place only after all movement ceases and the animals are greatly changed in shape and are dead. The same is true of ciliated trochophores.

2. Protoplasmic rotation in *Elodea* ceases simultaneously with decolorization in $n/40$ NH_4OH . In $n/40$ NaOH protoplasmic rotation ceases much before decolorization occurs.

3. Muscular contraction and nerve conduction in *Cassiopea xamachana*, a jelly-fish, continue *after* the red-stained granules of the ectoderm have been completely decolorized by $n/250$ NH_4OH , but in $n/250$ NaOH contraction and conduction cease *before* these granules are affected.

According to Warburg³ rate of oxidation in sea urchins' eggs is markedly increased by concentrations of NaOH which do not enter, while no increase in rate of oxidation is brought about by concentrations of NH_4OH which enter instantly.

The difference between NaOH and NH_4OH is very marked, whatever the type of cell studied, plant or animal, marine or fresh-water form. Judging from the color change of neutral red, NH_4OH enters instantly, without appreciably affecting cell activity, NaOH only after the cell is irreversibly affected — is dead.

The following conclusions may be drawn from the above facts:

1. The normal plasma membrane is impermeable to NaOH . 2. NaOH enters cells only after chemically changing the composition of the plasma membrane, making it permeable to NaOH . 3. Functional changes are induced by the effect of NaOH on the plasma membrane *before it has penetrated to the interior of the cell*. A verification of the latter conclusion would be of special interest in view of the existing controversy over the relative importance of the plasma membrane in cell activity.

³ WARBURG, O.: Zeitschrift für physiologische Chemie, 1910, lxvi, p. 305.

All depends upon the sensitivity of neutral red as a detector of alkali within the cell. Two questions present themselves: 1. Is the neutral red combination in the presence of proteins or lecithin just as sensitive for NaOH as for NH_4OH ? 2. Assuming that both NaOH and NH_4OH entered at the same rate, would they both decolorize the neutral red combination in the same concentration?

1. *Influence of proteids and lecithin.* — May not the proteins or lipoids (lecithin and cholesterin) exert some influence on the NaOH, so that although it enters the cell it fails to affect the indicator and thus the appearance of cell impermeability is given? Dead cells, or cells killed by heat or saturated chloroform water, lose their high degree of permeability to NaOH, but it is possible that death or chloroform treatment affects the proteins. Experiments have indeed shown that egg albumen will combine with NaOH and that combined alkali cannot be detected by neutral red. We can be certain, then, that the proteids within the cell must be saturated with alkali before the indicator is affected. But egg albumen will combine also with the same amount of NH_4OH . Why, then, the great difference between penetration of NaOH and NH_4OH into the cell, unless an actual difference in permeability for these two substances exists?

In order to make absolutely certain of the above point I have studied the permeability of artificial cells surrounded by membranes whose permeability for NaOH and NH_4OH was known to be the same. The diffusion rate of these two alkalies through tubes of agar-agar jelly colored with neutral red was found to be practically the same. NH_4OH diffuses slightly more rapidly in consequence of its slightly smaller molecular weight (35 as compared with 40 for NaOH). By tying collodion films (made on a mercury surface) over the ends of the agar tubes and by other methods it was found that both NaOH and NH_4OH diffuse at practically the same rate through collodion. Accordingly collodion membranes of test-tube form (made in the usual way) were filled with the "artificial cell contents" colored with neutral red, tied to a glass rod which served the purpose both of holder and stirrer, and immersed in the alkali whose penetration was to be studied. A typical experiment is as follows:

A saturated solution of Merck's powdered egg albumen in distilled water was colored with a few drops of $m/100$ neutral red and 10 c.c. placed in each of two collodion test-tubes. One tube (A) was immersed in 500 c.c.

$n/1000$ NaOH; the other (B) in 500 c.c. $n/1000$ NH_4OH . Both were stirred constantly. After ten minutes the contents of B had turned gradually yellow and in twelve minutes A was of the same shade as B.

Other observations gave identical times for the penetration of NaOH and NH_4OH . In this experiment we have the slow penetration of an alkali through a membrane into a protein solution just as in a living cell. The protein makes no discrimination between alkalis. NaOH can affect the neutral red even in the presence of protein as readily as can NH_4OH .

A similar experiment showed that lecithin also affects equally the color change by NaOH or NH_4OH . As stated above, the neutral red in the living cell is in combination with granules or with very weak acids. Accordingly, various substances were stained in neutral red and introduced along with proteins in order to determine the effect of the proteins on the decolorization of these suspended granules. In no case was it found that NH_4OH caused the color change before NaOH. The following mixtures were tried:

1. Potato-starch grains stained in neutral red and suspended in the egg albumen solution.⁴

2. One part lecithin suspension in water + one part $n/20$ NaOH saturated with caseinogen + two parts of a mixture of undiluted white and yolk from one egg. Neutral red was added and the whole neutralized with HCl.

3. One part egg white + one part of the vitellin + globulin granules of the immature flounder's egg. After adding neutral red the whole was neutralized with HCl. The vitellin + globulin granules stain deeply in neutral red.

The last two mixtures imitate closely the conditions within the living cell, yet in both cases the color change took place in the same time in NaOH as in NH_4OH . We must conclude, therefore, that although the presence of protein and lecithin may decrease the sensitivity of neutral red as compared with pure water, the effect is the same for NaOH as for NH_4OH .

2. Nature of the neutral red combination. — McClendon⁵ has suggested that those granules of marine eggs staining in neutral red are

⁴ The egg albumen almost completely removes the neutral red from the starch, so that this experiment is not conclusive.

⁵ McCLENDON, J. F.: *Loc. cit.*, pp. 459-460.

"lipoid" in nature, that NH_4OH dissolves in them readily, while NaOH does not, and that it is for this reason that NH_4OH *appears* to enter the egg more readily. In actuality both alkalies may penetrate the cell at the same rate, but the NaOH being insoluble in the red-stained lipoid granules cannot enter and decolorize them.

I endeavored to control my experiments on living cells by observations on the penetration of NaOH into cells killed in various ways, *e. g.*, by chloroform-saturated water. Chloroform-killed cells were found to be entered just as readily by NaOH as by NH_4OH . Their normal impermeability to NaOH was destroyed by chloroform. However, the red-stained granules of the sea urchin egg are broken up by chloroform. The loss of resistance, then, may be due to the effect of chloroform on the granules rather than on the cell membrane.

This objection, however, does not apply to *Spirogyra*. In this plant the neutral-red combination (a neutral-red tannate) is not affected by chloroform-saturated water, yet the plasma membrane must be, for the cell immediately loses its high resistance for NaOH . The color change occurs as rapidly as in the same molecular concentration of NH_4OH . It is certainly true that the plasma membrane of *Spirogyra* is very impermeable for NaOH and readily permeable for NH_4OH , a conclusion which must be extended to animal cells also in view of the identical permeability relations of plant and animal cells — a point emphasized by Overton and others.

Even if the red-staining granules of eggs were pure lecithin in nature, for which there is no evidence, sufficient water must be present in them to dissolve both NaOH and NH_4OH . Experiments show that the watery "myelin forms" of lecithin are readily penetrated by both NaOH and NH_4OH . If the granules are pure oil or fat, they would take up the neutral red in the yellow condition only.⁶ If they are a solution of lecithin in oil or fat solvents, for which there is likewise no evidence, McClendon's suggestion may be valid, as indicated by the experiments recorded below. They are probably lecithoproteid in nature. The diffusion of NaOH and NH_4OH into various proteid and organic bodies is also considered below.

Globules of any size suspended in water may be obtained by shaking chloroform, benzol, turpentine, or oils with protein solutions.

⁶ The centrifuge shows that the red-stained bodies are not oil. They are the heaviest granules in the cell and are thrown to the centrifugal pole.

The protein forms a condensation film at the oil-water surface, preventing subsequent fusion.⁷ Such globules accumulate neutral red from aqueous solution, but only in the yellow condition (as a base). If lecithin is previously dissolved in the chloroform or oil, the yellow neutral-red base will unite with it, giving a salt, red in color. It is the color change of this salt from red to yellow when the globules are placed in equivalent concentrations of NaOH and NH_4OH which has been studied. By shaking the required amount, lecithin-chloroform droplets may be obtained of a diameter similar to that of a sea urchin's egg or even smaller. The chloroform is soon replaced by water and the water-lecithin globules or "cells" then resemble sea urchin's eggs to a remarkable degree.⁸

Globules of lecithin in various solvents were prepared and stained by shaking the lecithin solution with dilute egg albumen solution plus neutral red. The globules (about 0.1 mm. in diameter) were then placed in dilute ($n/100$ to $n/1000$) NaOH and NH_4OH . If the solvent for lecithin is benzol, toluol, xylol, turpentine, carbon disulphide or carbon tetrachloride, the globules are entered instantly by NH_4OH but not by NaOH. The NaOH does not enter benzol droplets in the course of an hour, even if the concentration is one tenth normal. Such a result corresponds to McClendon's hypothetical condition in the cell.

If chloroform is used for solvent, NH_4OH enters the globules more rapidly than NaOH when first prepared, but not later. The chloroform is gradually replaced by water, and the water-swollen lecithin within as well as the proteid film is penetrated at the same rate by NH_4OH and NaOH.

If the solvent for lecithin is ether, ethyl acetate, butyrate, propionate, or valerianate, resistant proteid films do not form about the globules, and the lecithin appears at the surface as red-stained myelin forms. These are decolorized rapidly and at the same rate by both NH_4OH and NaOH.

Cholesterin cannot be studied, since neither the crystals nor a solution in chloroform or benzol is colored by neutral red. The dye is taken up by the solvent only in the yellow condition.

⁷ ROBERTSON, T. B.: *Journal of biological chemistry*, 1908, iv, p. 1.

⁸ HARVEY, E. N.: *Science*, 1912, p. 564; and *Biochemical bulletin*, 1912, ii, p. 52.

In this connection it is interesting to note that lecithin itself is equally well penetrated by NaOH and NH_4OH , but not a solution of lecithin in benzol. If lipid solubility is to be accepted as the test of cell penetrability, the evidence from a study of alkalies indicates that the surface of the cell is not lecithin but benzol. Indeed the behavior of the cell toward dyes, alkaloids, and alkalies suggests that it is surrounded by a film of some fat solvent rather than one of lecithin — a condition manifestly impossible. Whatever the mechanism of entrance of a substance, the fact remains that those substances readily soluble in *fat solvents* penetrate cells easily.

McClendon's objection to the use of neutral red as an indicator would hold only if the red-stained granules within cells were composed of some substance like benzol or oil in which water does not dissolve. Unfortunately the red-stained granules of the sea urchin's egg or of *Paramoecium* break down and lose their dye when the cell is crushed, so that it is impossible to test their behavior toward NH_4OH and NaOH directly. I have, however, determined the rate of penetration of these two alkalies into various types of protein granules and crystals, besides other substances, to see if any difference between them would appear.

Potato starch grains, corn starch grains, lecithin globules, colloidal particles, vitellin granules from hen's egg yolk, and the granules precipitated from an extract of immature flounder's eggs by distilled water (probably mainly lecithoproteid), when stained in neutral red, are all turned yellow by very weak ($n/1000$ to $n/4000$) NaOH and NH_4OH at the same rate. Edestin crystals from hemp seed are dissolved and decolorized more readily by $n/2000$ NaOH than NH_4OH . Caseinogen particles turn yellow only when they swell and dissolve in alkali. Solution occurs at approximately the same rate in NaOH and NH_4OH . Oleic acid globules turn yellow in alkali only when soap is formed. Soap is formed more rapidly in NaOH.

Thus a large number of small particles, some of them lecithoproteid in nature, are found to take up neutral red and the combination formed to be decolorized as readily or more readily by NaOH than by NH_4OH . We can safely conclude, I think, that the similar granules within animal cells would behave in the same way. Although the neutral red within the cell is not so sensitive to alkali as in water, never-

theless neither proteins, lecithin, nor the nature of the neutral-red combination affects the degree of sensitivity for different alkalies. The indicator method for detection of alkali within the cell is therefore a perfectly adequate one, and the above-mentioned conclusions which I have drawn from permeability studies seem perfectly justified.

NEW GALVANIC PHENOMENA.

By REINHARD BEUTNER.

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AS is well known, a galvanic cell consists in general of two different metals which are immersed in a salt solution. An arrangement of this kind will give rise to an electric current if the two metals or poles of the cell are connected by a wire. The investigation of these phenomena by Volta, after Galvani's first and famous discovery, now more than a century ago, marks the beginning of a rapid progress of our knowledge of electric phenomena.

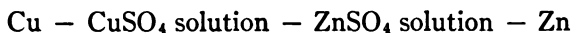
About fifty years later, it was found that organic tissues also give rise to an electric current. These currents were most extensively studied by DuBois-Reymond, who was hopeful to find new important facts concerning the physical nature of vital phenomena in general through his investigations. However, as he admitted himself, he did not succeed very well in this regard; he even failed to find a satisfactory physical explanation for the generation of electric currents by tissues. This was perhaps not so much due to an insufficiency of his physiological methods, but rather because the physics of his time did not show him how to carry out his investigations thoroughly enough to go into the details of the complicated electric phenomena in tissues. No other galvanic cells than those with metal electrodes were known to give rise to a current equal in magnitude to that of organic tissues. No metals or even metal-like substances can, however, possibly occur in tissues.

The research work done after DuBois-Reymond chiefly aimed to establish the relations between electric properties of tissues and vital phenomena. Concerning the physical nature of the biological currents, some new hypotheses were put forward, which, however, could not be proved experimentally.¹

¹ *I. e.*, Ostwald's theory of ionic permeability of tissue membranes. Experiments of Tammann and Walden (both independent of each other) have proved

The new galvanic phenomena which I shall describe here relate to cells of a very different type from those known so far, as they are built up from water-immiscible² organic liquids and aqueous solutions. It is easy to see that an investigation with such cells may well serve to solve just those problems which DuBois-Reymond left unanswered. I can prove so far, that these new cells exhibit in a similar way some electric properties of tissues which were first observed by Dr. J. Loeb and myself last winter.

Before I explain these relations I wish to repeat first some of the more general facts about galvanic phenomena. It has been proved beyond doubt by the famous investigations of Helmholtz that the existence of a galvanic current is a thermodynamic necessity; this means that such a current must exist, if the law of the conservation of energy is true. A plain example may serve to make the matter clearer. It is known that an electric current, passing through water or certain aqueous solutions, decomposes the water into oxygen and hydrogen. The oxygen-hydrogen mixture produced represents a certain amount of energy, for it can be used either to drive a gas engine, *i. e.*, to produce mechanical work, or the energy can be transformed into heat by an explosion. This amount of energy cannot be gained from nothing; it has been taken from the current. In order to bring about the decomposition, the current, if passing through the water, has to overcome an opposing force, the so-called "polarization" or "electromotive force." In the case just discussed, the circumstance that the products of decomposition are gases and escape quickly from the electrodes intervenes. If, however, we pass a current from an outside source through a Daniell cell



it will also cause a chemical decomposition (precipitation of Zn metal from the ZnSO₄ solution and solution of Cu metal, when the current passes from the Cu to the Zn), but in this case the products of decomposition are identical with the material of the electrodes, and the

that the conception of an ionic permeability is contradictory to experimental observations for artificial membranes. Haber's theory that the membranes behave like hydrogen electrodes has been proved to be contradictory to fact by Dr. J. Loeb and the writer (Science, 1911, vol. xxxiv. p. 886).

² *I. e.*, immiscible with water in any ratio.

change brought about by the current is only a quantitative one. The current sent through this system from an outside source has therefore to overcome an opposing electromotive force in the same way. This electromotive force, however, exists also in absence of an external source and will generate a current by itself if the poles are connected.

The magnitude of a current generated by a cell depends on many unessential circumstances, like the size of the vessel which contains the cell and the resistance of the connecting wire. The electromotive force is that magnitude which is really characteristic for a definite cell arrangement, so far as the chemical composition is concerned; this electromotive force must be measured while no current is passing through the system, *i. e.*, by an electrometer, that is, an instrument the turns of which are due to electrostatic attraction.

As an electromotive force depends on a chemical reaction it is subject to the same influences as a reaction. For example, the influence of temperature upon the electromotive force has been calculated from chemical thermodynamic theories and experimentally verified.

As is well known, the concentration of a dissolved compound has a great influence upon its chemical activity. Therefore the electromotive force depends also on concentration (for instance, if we dilute the copper sulphate solution in a Daniell cell, the electromotive force decreases). Nernst, in well-known investigations on this property of electromotive force, derived a formula which states that the change of the electromotive force is proportional to the logarithm of the ionic concentration. This so-called Nernst's law, which can be easily verified by experiments, is a specialization of the thermodynamic theory of Helmholtz and of the theory of electrolytic dissociation of Arrhenius. Nernst, however, made the conception of Helmholtz more conspicuous by pointing out that the electromotive force of a cell is (mainly) composed of so-called "potential differences" at the junction of metal and solution, and that these potential differences vary regularly if the concentration is changed.

Dr. J. Loeb and the writer investigated the electromotive force of tissues a year ago.³ DuBois-Reymond and most other physiologists, who worked along this line, had used animal tissues as physiological objects, mostly frog muscles. Dr. Loeb, on the contrary, suggested the use of parts of plants for the reason that they possess a far greater

³ Science, 1911, xxxiv, pp. 884-887.

chemical constancy. This was of great importance for our investigations, as it was found possible with plants only to study the very remarkable electromotive properties of tissues, while the electromotive force of animal tissues is so inconstant owing to chemical decomposition that an accurate measurement and application of physical laws appeared impossible.

It was found in this investigation ⁴ that the electromotive force of a cell arrangement which contains a piece of plant tissue as a "middle conductor" ⁵ exhibits regular and reversible changes, if the concentration of a solution in contact with the skin of the plant is varied. The magnitude of the change was about such as could be expected from Nernst's formula. This proves that the change of the electromotive force must be located at the junction of the plant skin and the aqueous solution of varying concentration. ⁶ This junction is the seat of a potential difference in the definition of Nernst, and behaves in fact similarly as the junction of a metal and a solution. There is, however, a different behavior inasmuch as with metals only a solution of a salt of the metal itself has any effect at all (*i. e.*, with silver electrodes only the concentration of a silver salt in the solution, addition of copper or zinc salts has no influence at all). The potential differences at the junction of tissues, however, vary with the concentration in the same way whether a KCl or a CaCl₂ or Na₂SO₄ solution is used. A solution of any (non-poisonous) electrolyte acts in the same fashion.

An explanation of these electromotive properties of tissues can be found through the following merely physical investigations which the writer has carried out. Water-immiscible organic liquids which contain water-insoluble acids act in the same manner as the skin of plants. As an example I wish to cite here my measurements in this system:

⁴ Concerning the experimental arrangement used, *cf. loc. cit.*

⁵ This means that the arrangement is such that the electric conduction must take place through the tissue.

⁶ It is known that the junction of two aqueous solutions is also the seat of a potential difference which varies with the concentration. Such so-called diffusion potentials necessarily also occur in the "cell" arrangement of Dr. Loeb and the writer. Their magnitude, however, as well as the magnitude of their variation with concentration, is by far too small to account for the change of the electromotive force observed. The same holds for the "cells" built up from water-insoluble organic substances and aqueous solutions.

Hg — $n/10$ KCl solution saturated with calomel — salicylic aldehyde saturated with salicylic acid — (KCl solution of varying concentration) — $n/10$ KCl solution saturated on calomel — Hg.

The electromotive force of this system (measured with Dolezalek's electrometer) varied according to the concentration of the KCl solution (in parentheses) as the following figures show.⁷

TABLE I.

Time in minutes.	Concentration of the variable KCl solution. (In gram-molecules in a litre.)	E. m. f. observed in millivolts.	Difference of the e. m. f.'s.	Time in minutes.	Concentration of the variable KCl solution. (In gram-molecules in a litre.)	E. m. f. observed in millivolts.	Difference of the e. m. f.'s.
0	1/10	5	...	73	1/250	53	
1	1/10	6		78	1/50	25	28
3	1/50	30	24	81	1/50	24	
4	1/50	30		84	1/10	0	24
7	1/250	55	25	86	1/10	0	
9	1/250	55		88	1/2	— 21	21
11	1/1250	89	34	90	1/2	— 21	
12	1/1250	89		95	2 1/2	— 39	18
17	1/6250	130	41	97	2 1/2	— 40	
19	1/6250	130		103	1/2	— 22	18
20	1/1250	89	41	104	1/2	— 22	
23	1/1250	88		107	1/10	0	22
26	1/250	54	34	108	1/10	0	

These measurements, which extend over a period of nearly two hours, show to what degree of accuracy measurements of this kind may be carried out. The fact that the electromotive force of the system comes back very nearly to the same value if the same concentration

⁷ Concerning the physico-chemical technique it may be said that "liquid potentials" cannot intervene with these measurements, as the migration velocity of K^+ and Cl^- is alike.

is applied again proves that these measurements are far from being accidental. Comparing these electromotive forces with those observed by Dr. J. Loeb and the writer on apples,⁸ the identical change of the electromotive forces in both cases is seen in a striking way. Instead of a KCl solution, solutions of various other salts were employed with a similar result: NaCl, NaNO₃, Na-Acetate, Na₂HPO₄, Na₂SO₄, CaCl₂, NH₄Cl, CuSO₄. These electromotive forces depend, like those of tissues, on the concentration of various salts.

Instead of the salicylic aldehyde-salicylic acid mixture various other water-immiscible organic liquids were employed. The proof that the strong acid character of the salicylic acid is responsible for these electromotive forces is found in the fact that kresol or phenol or other water-insoluble liquids of a less pronounced acid character do not show a change of electromotive force with the concentration. This is also seen from the fact that benzoic acid dissolved in benzaldehyde acted like the salicylic aldehyde-salicylic acid mixture, but the variation of potential difference is much smaller in this case; benzoic acid, in fact, is a much weaker acid than salicylic acid.⁹

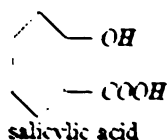
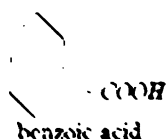
Furthermore, I have found that water-immiscible bases exhibit a change of the potential difference in the opposite direction, as water-immiscible acid liquids, *i. e.*, aniline, toluidine, methylaniline, naphthylamine, and various other bases were studied. The electromotive force of a cell like

Hg — *m*/10 KCl solution saturated on HgCl — toluidine — (KCl solution of varying concentration) — *m*/10 KCl solution saturated on HgCl — Hg

varies in the opposite direction with the concentration of the KCl solution (in parentheses) as does the electromotive force of the arrangement described before, but to nearly the same extent and quite as regularly. Instead of KCl various other salts may also be employed with the same results.

⁸ Compare Table I, *Loc. cit.*; Science, 1911, xxxiv, p. 885.

⁹ This is seen from Ostwald's "Affinitätskonstanten." Also the chemical constitution of the two acids accounts for the same fact.



The OH group of the salicylic acid is essential for the stronger acid properties.

From water-immiscible acid and basic organic substances the new galvanic cells are built up which I have mentioned in the beginning. I wish to cite here a few examples of such cells.

- (1) $\text{—Hg—}n/10 \text{ KCl sol. — salic. ald. — }m/10 \text{ HgCl}_2 \text{ sol. — }n/10 \text{ KCl sol. — Hg+}$
 (sat. on HgCl) (sat. on HgCl)
 electromotive force: 0.14 volt.
- (2) $\text{—Hg—}n/10 \text{ KCl sol. — salic. ald. — }m/10 \text{ MgSO}_4 \text{ — }m/10 \text{ KCl — Hg+}$
 electromotive force: 0.086 volt.
- (3) $\text{+ Hg — }m/10 \text{ KCl — }n/10 \text{ KSCN — toluidine — }m/10 \text{ Na}_2\text{SO}_4 \text{ —}$
 $n/10 \text{ KCl — Hg —}$
 electromotive force: 0.25 volt.
- (4) $\text{+ Hg — }n/10 \text{ KCl — toluidine — }m/10 \text{ Na}_2\text{HPO}_4 \text{ — }m/10 \text{ KCl — Hg —}$
 electromotive force: 0.116 volt.
- (5) $\text{+ Hg — }n/10 \text{ KCl — toluidine — }n/1250 \text{ KCl — salicyl. ald. —}$
 $n/10 \text{ KCl — Hg —}$
 electromotive force: 0.19 volt.

(This last system is a new type of "double concentration cells.")

The electromotive force of some of these cells is even considerably higher than that of tissues in general.¹⁰ The magnitude of the electromotive force produced by living organs, which so far seemed to be unaccountable by experiments, can no more appear as a "vital" mystery.

As was said above, the electromotive force of a galvanic cell is determined by the chemical reaction brought about by a current passing through the system. If we know the nature of this reaction for a definite cell, we have reached the most perfect explanation possible. For the new galvanic cells described above, the solution of this problem has been possible to some extent. My methods, however, to determine these reactions cannot be described in full here because this discussion involves too many questions of a special physical nature. I wish to add only that the thorough experimental and mathematical investigation of the relation between concentration and potential

¹⁰ "Liquid potentials" can only influence the electromotive force of these systems very slightly. The metallic Hg-electrodes do not produce even the least part of the electromotive force, as they are alike and opposite (the $n/10$ KCl always being saturated on HgCl).

difference which was discussed above, is a most important step towards the solution of this problem. My mathematical theory concerning this relation is based upon the physico-chemical laws of mass action and distribution, and has been verified by experiment.

The result of the theory is expressed as follows:

$$\text{potent. diff.} = \frac{RT}{nF} \ln \left(\frac{1}{2C} + \sqrt{\frac{1}{4C^2} + K + \frac{M - K}{C}} \right) \cdot \text{const}$$

In this formula R is the gas constant, T the absolute temperature, n the valency of the kation, F Faraday's equivalent, "const" an integration constant. C is the concentration, M and K are calculated through the following relations:

$$M = \frac{1}{b^2 m^2} - \frac{1}{abm}$$

where a and b are special values of C ; m is determined experimentally through this relation:

$$\text{pot. diff. } (C = b) - \text{pot. diff. } (C = a) = \frac{RT}{nF} \ln m$$

Furthermore, if d is a third arbitrary value of C , m' is determined as

$$\text{pot. diff. } (C = d) - \text{pot. diff. } (C = a) = \frac{RT}{nF} \ln m'$$

we express M' as

$$M' = m' \left(\frac{1}{2a} + \sqrt{\frac{1}{4a^2} - M} \right)$$

Through these magnitudes K (in the main formula) is found by this relation:

$$K = \frac{1}{1 - \frac{1}{d}} \left(M'^2 - \frac{M'}{d} - \frac{Ma}{d} \right)$$

The application of the formula, *i. e.*, to the measurements cited in Table I, gives the following result:

Ratio of concentrations.	Change of the potential difference.	
	Calculated.	Observed.
	<small>milli-volts</small>	<small>milli-volts</small>
1/2 over 1/10	24	21
1/10 over 1/50	22	24
1/50 over 1/250	24	28
1/250 over 1/1250	29	34
1/1250 over 1/6250	34	41

Owing to the merely physical character of this communication, the detailed biological application of the new galvanic phenomena is not to be discussed here. It seems that at this time even the physical investigation along this line is too much in its infancy, and that more details should be known in order to make a satisfactory application possible.

When our knowledge concerning electric properties of organic substances and organic tissues will be more advanced than at present, it seems likely that electric measurements will furnish a means to solve some questions concerning the physical nature of vital phenomena. Work along this line has been done by Bernstein and others, who proposed electric theories of muscular contraction, nervous excitement, etc. On account of the relations which exist between currents and chemical reactions, it seems possible that there exist some relations between electric currents in tissues and metabolism. Theories of this kind could be formulated more distinctly if the physical nature of bio-electric currents were known more accurately.

THE RELATION OF VENOUS PRESSURE TO CARDIAC EFFICIENCY.¹

By VANDELL HENDERSON AND THEODORE B. BARRINGER, JR.

[From the Physiological Laboratory of the Yale Medical School.]

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I. THE DIASTOLIC FILLING OF THE VENTRICLES.

THE volume of blood which the ventricles throw out during systole is determined by the volume which runs into them during diastole. The idea formerly held that the ventricles during relaxation exert a suction and thus to some extent refill themselves has been shown by von den Velden² to be erroneous. The observations to be reported here demonstrate in addition that a distinct pressure in the venous stream is needed to expand the heart during the diastolic relaxation.

The relaxation of the heart is not, however, a mere mechanical process, like the stretching of rubber, which may be quick or slow according to the character of the force applied. On the contrary, its relaxation, like that of any other muscle, depends upon vital processes (oxidation) to the same degree as does its contraction. A heart

¹ In a previous paper we have discussed the general subject, namely, the conditions determining the volume of the arterial blood stream, one phase of which is here considered. HENDERSON and BARRINGER: this Journal, 1913, **xxxi**, p. 288.

² R. VON DEN VELDEN: Zentralblatt für Physiologie, 1906, **xx**, p. 73; also Zeitschrift für experimentelle Pathologie und Therapie, 1906, **iii**, p. 432.

which relaxes slowly can fill only slowly. In such a heart a brief diastole (*i. e.*, a rapid rate of beat) necessarily involves a small amplitude of stroke. On the other hand, a heart which relaxes and fills quickly may maintain a fairly large systolic discharge even at a rapid rate of beat.

For maximal cardiac efficiency it is essential also that the venous supply should be sufficient, and at a sufficient pressure, to distend the ventricles as rapidly as the diastolic relaxation of the myocardium allows.³ From these considerations it follows that the volume of blood entering the ventricles during any diastole (and in consequence the volume discharged by the next systole) depends upon two sets of conditions: (1) *the inherent rate and extent of relaxation of the myocardium*; and (2) *the volume and pressure of the venous stream to the right heart*.

The first of these factors was carefully investigated in this laboratory some years ago.⁴ It was found that, under experimental conditions as nearly normal as possible, the ventricles at all rates of beat obey a principle of "uniformity of behavior." As the present paper is partly a study of deviations from this principle, it may advantageously be restated here as a standard of comparison. By means of it we can analyze the behavior of the heart at various rates of beat and under different venous pressures, so as to determine the influence of the latter factor independently of the former. Without this method of analysis it would be quite impossible to decide how much of an increase or decrease in the systolic discharge is due to altered rate of beat and how much to variations in venous pressure.

The principle is illustrated in the four schematic ventricular volume curves in Fig. 1. Suppose that at a slow rate of beat the heart contracts and relaxes so as to afford a volume record consisting of a series of such curves as the first (at the left). Then at more rapid rates the volume record is always normally composed of such strokes as those expressed by the solid lines in the second and third curves. When the curve of one of the slower fuller beats is superimposed (as shown by the dotted lines) upon one of those at the more rapid rates, *the smaller are found to consist of abbreviated arcs of the larger*. This

³ This is strikingly shown in some of the curves obtained by WIGGERS, C. J.: Archives of internal medicine, 1910, vi, p. 281.

⁴ HENDERSON, Y.: this Journal, 1909, xxiii, p. 345.

relation holds true, however, only so long as the heart is beating with full vigor and the blood is circulating with normal velocity. The first indication of circulatory failure is a deviation in the volume curve such as that shown in the solid lines of the fourth curve (at the right). Here the more gradual up-stroke indicates that the diastolic filling of the ventricles is less rapid than normally. Consequently the ampli-

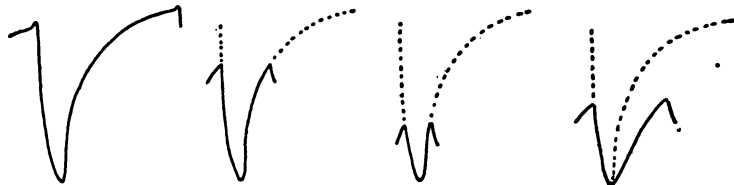


FIGURE 1.

tude of stroke and volume of systolic discharge are only a little more than half as large as the heart is capable of making at that rate of beat.

The foregoing statement takes no account of auricular systole. In deriving the normal amplitude of beat for any rate from the "complete volume curve" it is not necessary to do so. There is indeed sometimes (and especially when the plethysmograph is badly adjusted upon the ventricles) a considerable rise of the ventricular volume curve coincidently with auricular systole, but with a vigorous heart and adequate venous pressure the curve always falls back to the ordinary relaxation line immediately thereafter. This indicates that the contraction of the auricle has caused a wave to pass over the blood in the ventricle,⁵ but that it has not increased its volume. It is only when the venous supply and pressure are inadequate for full cardiac efficiency that the ventricles sometimes fill in two distinct stages, one early in diastole and the other coincident with auricular systole (*cf.* last two sections of Fig. 3, and before saline infusion in Fig. 6).

II. THE MEASUREMENT OF VENOUS PRESSURE.

Experiments were made upon twenty dogs. In addition to the procedures described in our previous paper for maintaining a natural respiration of slightly compressed air, and for recording arterial pressure and the cardiac volume curve, a special technique was employed for the measurement of venous pressure. To make these measurements

⁵ HENDERSON and JOHNSON: *Heart*, 1912, iv, p. 77.

reliable was found to be far more difficult than previous investigators seem to have realized. The uncertainty is due not merely to valves and clots, but rather to the fact that the slightest compression or stretching of the tissues through which the vein passes is sufficient to collapse it. The reading on the manometer may thus easily be elevated many centimetres above the true pressure, or depressed as much, without the observer being led to suspect the error. A criterion is needed. It was found in the form of manometer and arrangement shown in Fig. 2. The right jugular was dissected out down to its junction with the subclavian and up to the level of the larynx, where it was cut between double ligatures. The manometer was inserted on the side toward the heart, and was then lifted so that the stump of the jugular (2 or 3 cm. in length) was drawn straight upward at its junction with the subclavian. Thus this stump itself formed the lower part of the manometer tube. Beneath it the stream from the subclavian into the vena cava flowed uninterruptedly, while the column of saline in the manometer prevented the blood from rising high enough to touch the glass. Clotting was thus usually avoided, and yet the fluid connection between the manometer and the heart was so free that the pulsations of the right auricle showed distinctly in the column of blood and saline. An additional criterion was obtained before each reading by opening for a moment the pinch cock at the side of the manometer so as to raise the height of the column of saline two or three centimetres. If thereafter the column fell quickly and stopped abruptly, the vertical distance between its top and the level of the ventral side of the auriculo-ventricular groove was immediately measured in millimetres. For the reasons stated in the next paragraph these measurements were corrected as follows: When the reading was 60 mm. or more, 10 mm. were subtracted. When it was 40 to 55 mm., 5 mm. were subtracted. When it was below 40, it was not reduced. The figures so corrected were taken as the pressure in millimetres of saline

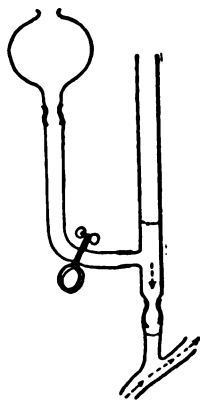


FIGURE 2. — Arrangement for measuring venous pressure. Subclavian vein, jugular, which has been cut and drawn vertically upward, manometer, and reservoir of saline. The lower meniscus shows the top of the column of blood, the upper that of the saline.

in the vena cava superior. Only 0.3 to 0.5 c.c. of saline was injected at each reading, a quantity of no importance for animals of the size employed.

Especial care was taken that the cardiac plethysmograph should not cause errors in the venous pressure readings. It was found that if the rubber curtain around the window fitted too tightly upon the auriculo-ventricular groove, it interfered with the filling of the ventricles and dammed the blood back in the veins. In order to avoid this error, the venous pressure was measured both immediately before and after the plethysmograph was placed upon the heart. If the second reading was more than 10 mm. higher than the first (it was seldom less than 5 mm.), the plethysmograph was removed and the opening in its window enlarged. It was then replaced and again tested. Even with this precaution obstruction was liable to result if later on the heart swelled owing to a decreased tonus. Accordingly at the beginning of each experiment and until this adjustment had been made, the pulmonary ventilation was restricted, and the heart was thus regulated to a slow rate of beat and low tonus, *i. e.*, relaxed and largely distended ventricles. Throughout these experiments the method of regulating the heart rate and tonus by varying the pulmonary ventilation was employed.⁶

III. THE RELATION OF RATE OF FILLING TO VOLUME OF DISCHARGE.

In considering the venous pressures in these experiments it is essential to keep in mind that the thorax was open, and that the heart and thoracic veins were thus deprived of the influence of the negative pressure due to the elasticity of the lungs. By the opening of the pleural spaces the heart was, so to speak, lifted out of the valley into which the venous stream normally drops. Pressures in the vena cava superior of +10, zero, and -10 mm., in the unopened thorax with an intrapleural negative pressure of -50 mm. would have the same influence upon the filling of the heart as pressures of +60, +50, and +40 mm. respectively after pneumothorax. The generally accepted figures for the thoracic negative pressure are about -45 to -70 mm. of water during expiration and -100 mm. or more during inspira-

⁶ HENDERSON, Y.: this Journal, 1908, xxi, pp. 147 and 153.

tion.⁷ These figures express the depth of the thoracic valley at the bottom of which is the heart. Now it is only after hemorrhage or in some degree of shock that the top of the venous column (in a recumbent subject) falls below the edge of the valley. The jugular just above the clavicle is then collapsed. In the normal subject it rises above the edge. In other words, *the pressure in the thoracic veins during all phases of quiet breathing is normally slightly above atmospheric pressure*, for we have observed that the jugular veins of a normal dog are constantly distended by a distinct positive pressure (5 to 10 mm. of saline or more).⁸ That this is true also of normal men in the recumbent position we shall show in a later section (VI). Thus the conclusion seems justified that in the normal subject the intrathoracic venous column, *i. e.*, the force available to distend the right ventricle during diastole, is normally never less than 45 mm. of water and is usually much more. During a deep inspiration the pressure in the thoracic veins becomes negative and the jugulars collapse; but even then the top of the column falls no more, and probably no less, than does the bottom of the thoracic valley. The effective pressure remains the same. In what follows it will be seen that what we shall call *the critical venous pressure necessary to distend the right ventricle as rapidly as it relaxes is not more than 50 mm. of saline.*

The thoracic venous pressure is expended partly in driving the blood through the tricuspid orifice, and partly in pushing the ventricular walls outward. The amount of pressure needed for the former purpose may be roughly estimated from the equations:

$$2A^3 = A^2 \times C \times T \times \sqrt{2g(Ha - Hv)}$$

$$\text{or} \quad Ha - Hv = \left(\frac{2A^3}{A^2 \times T \times C} \right)^2 + 2g.$$

In these equations $2A^3$ is the systolic discharge or tidal blood for which a volume of 2 c.c. per kilo body weight for small animals and 1 c.c. per kilo for a man are approximate values. A^2 is the cross section of the tricuspid orifice. C is its coefficient of discharge, probably about unity. T is the

⁷ After the thorax was opened it was necessary to maintain a pressure of 60 to 100 mm. in the air supply in order to keep the lungs distended sufficiently for spontaneous breathing.

⁸ BURTON-OPITZ (this Journal, 1902, vii, p. 446, and 1903, ix, p. 201) found lower figures, but they merely indicate that the circulation had begun to fail.

duration of the period of rapid filling early in diastole, 0.1 second for small animals and probably 0.3 second or more for man. $H_a - H_v$ is the difference in pressure between auricle and ventricle in millimetres of blood (or saline). On solving the second equation with probable values for subjects of various sizes from small dogs up to man, we find that $H_a - H_v$ need not be more than 15 mm. Accordingly, when the difference between venous pressure and intra-pleural pressure is 50 mm., a force of 35 mm. is available to force the ventricular walls outward during the diastolic relaxation.

In Fig. 3 are reproduced some of the volume curves (lower record) and the simultaneous venous pressure measurements from a large dog. The first section of the record shows three of the large slow beats occurring during expiration followed by three quicker and somewhat smaller beats during inspiration. The venous pressures at the time of the third and sixth beats were 60 and 45 mm. of saline respectively. Careful comparison of the volume curves of these two beats shows that they are not quite superimposable. In the former the up-stroke of the volume curve is slightly steeper than in the latter. This indicates that under the influence of a venous pressure of 60 mm. the diastole inflow and distension of the ventricle was a little more rapid than with a pressure of only 45 mm. The systolic discharges of the first four beats were larger than the last two, partly because of this more rapid filling and not merely because of the longer diastoles preceding and the slightly lower tonus accompanying them.

Soon after the foregoing observations had been made the animal was bled until the venous pressure was reduced to 35 mm. The second section of Fig. 3 was then recorded. The volume curves obtained fall far short of being superimposable upon those prior to the hemorrhage. Although the rate of beat was nearly the same as in the last three beats of the first section of the record, the up-strokes of the curves are so much less steep that the amplitude of the strokes (*i. e.*, the systolic discharge) was a third less with a venous pressure of 35 mm. than with one of 45.

After a second and a third hemorrhage the venous pressure was lowered to 20 and finally to 10 mm. The slower filling of the ventricles during diastole and the consequent decrease of the systolic discharges are strikingly shown in the third and fourth sections of Fig. 3. From this experiment as a whole it appears, therefore, that with venous pressures below 60 mm. and especially below 45 mm. of saline

the systolic discharge is progressively diminished. Between 45 and 60 mm. the difference is, however, slight. The question thus presents itself whether at higher venous pressures the rapidity of filling and the volume of the systolic discharge is further increased.



FIGURE 3.—Two fifths the original size. From a dog of 18 kilos. Time in seconds. Arterial pressure. Ventricular volume curve. The first three beats occurred during expiration, the next three during inspiration. Below the curves are noted the (corrected) venous pressures in millimetres of saline. Note that the volume curves of the last three strokes in the first section are not exactly superimposable upon the first three. The up-strokes are less steep; *i. e.*, the diastolic filling is slower, because of the lower venous pressure. In the second, third, and fourth sections the venous pressures are progressively lower because of hemorrhage. The filling of the ventricle is correspondingly slower and the strokes smaller. At no time during the experiment was the cardiometer opened or removed. The tonus changes of the heart are therefore correctly indicated by the lowering of the volume curve as a whole.

In Fig. 4 are reproduced curves and measurements which indicate clearly that (at least in this case) the efficiency of the heart is practically the same at all venous pressures above 50 mm. In the first two sections of this record marked variations in rate of beat occur synchronously with the animal's breathing. In the first section venous pressure rose during the slow pulses of expiration to 90 mm., and fell with the accelerated beats of inspiration to 75 mm. In the second section the corresponding pressures were 65 and 50 mm. respectively. If now the curves at these four levels of venous pressure are compared, it is found that they are all, as exactly as the comparison can be made, superimposable. On the other hand, in the third section of the record when the venous pressure was 40 mm. the volume curves exhibit a distinctly slower up-stroke. Owing to this slower diastolic filling, the down-strokes or systolic discharges are also diminished. Similarly in the fourth and fifth sections of the record,

when the venous pressure had fallen to 20 mm. and then to 10 mm., the amplitude of the volume curves was progressively reduced.

It thus appears from this experiment, and from many similar experiments in close agreement with it, that (1) *at venous pressures below 50 mm. of saline the efficiency of the heart varies with the pressure.*

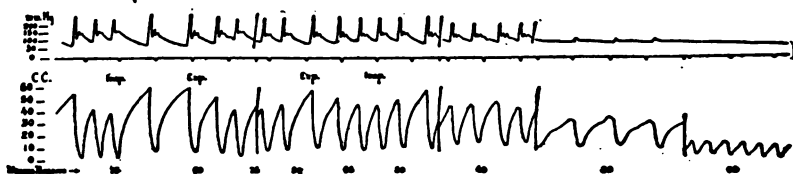


FIGURE 4. — About one fourth the original size. From a dog of 11.5 kilos. Arrangement as in Fig. 3. Note that at venous pressures of 90, 75, 65, and 50 mm. the volume curves are all superimposable, and that the amplitude of the strokes is therefore entirely determined by the duration of the diastoles. The rate of heat varied with the respiration: *Insp.* indicates inspiration, and *Exp.* expiration. At venous pressures of 40, 20, and 10 mm. the ventricles fill less rapidly, and the strokes are thus progressively decreased. In this series of records the tonus changes are not shown, as the plethysmograph was repeatedly removed in order that the correctness of the venous pressure measurements might be tested.

(2) *At what may be called the critical venous pressure of 50 mm. the heart beats with an efficiency which is maximal for each rate of beat.* And (3) *at pressures above 50 mm. the amplitude of beat is not increased by increase of venous pressure.*

These conclusions are further supported by four experiments in which the volume curve was recorded at venous pressures ranging from 50 mm. up to 100 mm., and in one case even up to 140 mm. These abnormally high pressures were never attained except at the beginning of an experiment, and even then only by a combination of moderate hypercapnia, saline infusion, and pressure upon the abdomen. In Fig. 5 is reproduced a part of the record of one of these experiments. Comparison of the volume curves at a venous pressure of 140 mm. with those at a pressure of only 53 mm. shows them to be practically superimposable. The difference in the amplitude of the strokes in the first two sections of Fig. 5 is entirely due to the slower rate induced in the first by hypercapnia. On the other hand, the third and fourth sections show the decreased efficiency at lower venous pressures.

IV. THE INFLUENCE OF TONUS UPON THE DISTENSIBILITY OF THE HEART.

The relations of venous pressure and cardiac efficiency above described hold true only when the tonus of the heart is normal or nearly normal. Both hypotonus and hypertonus induce marked altera-

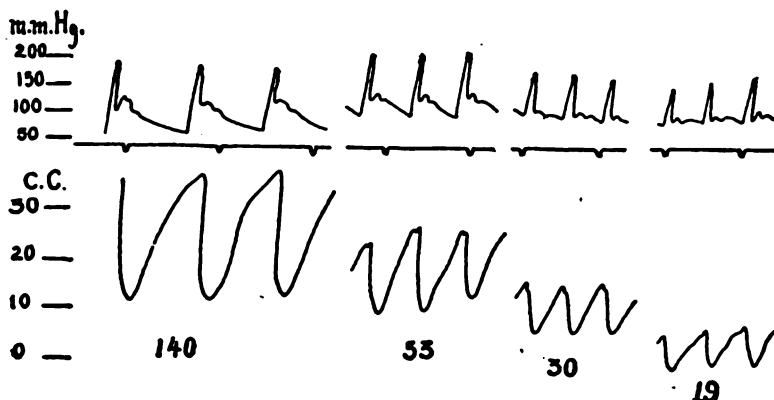


FIGURE 5. — Four fifths the original size. From a dog of 9.9 kilos. Arrangement as in Fig. 3. Note that at the enormous venous pressure of 140 mm. the efficiency is no greater than at 53 mm., for the volume curves in the second section are merely shorter arcs of the same curve as in the first. The difference in amplitude is entirely due to the difference in the duration of diastole, *i. e.*, the rate of beat. At venous pressures of 30 and 19, however, the shape of the curve is greatly altered and the efficiency correspondingly diminished. In this experiment the plethysmograph after its first adjustment was not removed from the heart. The tonus changes of the ventricles are therefore shown by the level of the volume curve. The greater the tonus, *i. e.*, the more contracted the heart, the lower the curve.

tions in the diastolic distensibility of the heart. Both conditions occurred frequently in our experiments. Both are abnormal, and unless extreme care in technique and critique is used are liable to become sources of erroneous observation and inference. Our entire experience indicates that the tonus of cardiac muscle is normally dependent mainly upon the rate of beat — in perfect similarity with the relation of tonus to rate of contraction in an excised striated muscle under a series of maximal stimuli. In addition the tonus of the heart is strikingly affected by the blood gases and the coronary blood supply. Variations thus induced are, of course, abnormal.

An abnormally high cardiac tonus is one of the common results of excessive pulmonary ventilation.⁹ The ventricles become so contracted that their cavities are practically obliterated, *i. e.*, completely emptied, at the end of each systole, while the extent of relaxation during diastole is small. This is due not only to the extreme tonus, but also to the tachycardia which accompanies it, and to the fact that the myocardium is much less readily distensible than normally. In some of our experiments the elevation of venous pressure even to a height of 100 mm. or more by means of saline infusion and abdominal pressure was insufficient to cause the ventricles to fill with normal rapidity. In some cases also we have noted such a contracture or cramp of the heart as one of the secondary effects of hemorrhage.

An abnormally low cardiac tonus developed whenever, in the attempt to induce and maintain an extremely slow heart rate, the pulmonary ventilation was reduced to the point of intense hypercapnia. The ventricles then became relaxed nearly or altogether to their maximum size during diastole, while only 55 to 65 per cent of their contents were discharged by systole. Their "systolic volume" or "residual blood" was thus about double the amount observed at ordinary degrees of tonus, and more than the "diastolic volume," *i. e.*, residual and tidal blood together, under abnormally high tonus. In hypercapnia with intact vagi the heart rate is always slow and venous pressure high, but a few observations after hemorrhage indicate that the atonic myocardium is more easily distensible than normally. In extreme low tonus it may even relax more rapidly than normally. The volume curves are thus sometimes better than superimposable, so to speak.¹⁰

V. FAILURE OF THE VENO-PRESSOR MECHANISM.

Most of the subjects of our experiments sank sooner or later into circulatory shock. This was the case even when there had been no considerable hemorrhage, and no direct injury to the nervous system. The succession of events was: (1) A progressive fall of venous pres-

⁹ HENDERSON, Y.: this Journal, 1908, xxi, p. 142; JERUSALEM and STARLING, Journal of physiology, 1910, xl, p. 279; HENDERSON and JOHNSON: Heart, 1912, iv, p. 78, footnote.

¹⁰ For volume curves in both extremes of tonus and all intermediate degrees, see HENDERSON, Y.: this Journal, 1909, xxiii, pp. 352, 365, 369.

sure. Meanwhile arterial pressure continued at a normal level, although the pulse became progressively smaller. (2) When venous pressure had fallen to a point at which the diastolic filling of the ventricles (and consequently the systolic discharge also) was reduced to a small fraction of the normal (a third or less), arterial pressure fell rapidly. (3) If no assistance was given, death ensued. If, however,

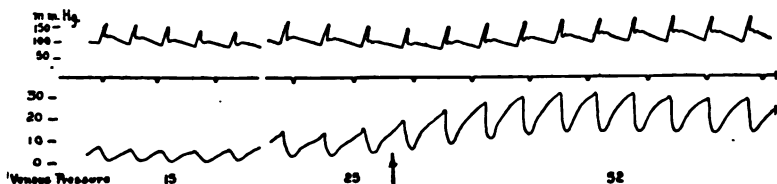


FIGURE 6. — Two fifths the original size. From a dog of 6.6 kilos. There had been no hemorrhage, but as the result of various operations (not involving direct injury to the central nervous system) shock was developing. Venous pressure had fallen to 15 mm., the amplitude of the heart beat was very small (only 5 c.c. per stroke), and arterial pressure would soon have fallen. Between the two portions of the record here shown the pulmonary ventilation was considerably decreased. A rise of venous pressure from 15 to 25 mm. resulted, causing the amplitude of the heart beat to be nearly doubled. Saline was infused (at the arrow), with the effects shown in the record.

before the failure had gone too far, saline was infused into a vein so as to afford an adequate supply to the right heart, the ventricles quickly recovered an almost normal amplitude of stroke and arterial pressure returned for a time to a normal level (see Fig. 6).

These facts indicate that circulatory shock is identical in its mechanics with the condition which we have shown to result from hemorrhage. In both the failure of the venous supply to the right heart is the critical factor.¹¹ They are quite distinct from the vasomotor failure and abolition of the peripheral resistance of the arterial system to be seen in spinal shock induced by section of the spinal cord.

VI. VENOUS PRESSURE IN THE NORMAL MAN.

Our observations have shown that in a heart with normal tonus the efficiency varies with the venous pressure at all pressures below 50 mm., but is maximal at this critical figure and all higher pressures. It be-

¹¹ HENDERSON, Y.: *this Journal*, 1910, *xvii*, p. 152.

comes important, therefore, to know whether the ordinary range of venous pressure in a healthy subject is above or below 50 mm. In a previous section (p. 357) we have stated the reasons why we believe that in the normal dog the conditions are such as to afford at all times an "effective pressure" (*i. e.*, the difference between intrapleural pressure and that within the great veins at the level of the heart) of more than 50 mm.

In the adult human heart it is probable that the larger size involves a somewhat greater critical pressure. The intrapleural or pericardial negative pressure is also somewhat greater, however, and it seems to us probable that if during passive expiration the pressure in the thoracic veins at the level of the heart is even slightly positive, the negative pressure of the chest would afford an effective pressure of at least the critical value. Proof that this condition is fulfilled is afforded by the thousands of venous pulse curves that have been recorded since Mackenzie introduced such records into clinical medicine. They show in every normal subject when recumbent a well-marked distension and pulsation in the external jugular veins just above the clavicle. It requires an extra deep inspiration to cause the vessels to collapse. During all phases of quiet breathing they remain distended. Each wave in the jugular is a demonstration of free and continuous fluid connection between the neck veins and the heart. As the flow through such wide vessels as the jugular and superior cava of man is subject to only an insignificant resistance, it follows that *within the thoracic veins of a normal subject in the recumbent position during quiet breathing the pressure is continually above that of the atmosphere, and the effective venous pressure is therefore of more than the critical value.*

It is extremely improbable that the circulation in a normal man is less when he is standing than when lying down. In spite of the hydrostatic disadvantage of the erect position we should expect the intrathoracic venous pressure at the level of the heart still to be positive (*i. e.*, above atmospheric pressure) by several millimetres of saline, if not by several centimetres. It is true that the jugular at the level of the clavicle is always collapsed in a man who is standing quietly; but this merely indicates that the venous pressure at the level of the heart is not sufficient to support a column of blood as high as the clavicle. Indeed the top of the column is below the axilla, for Gärtner¹²

¹² GÄRTNER: *Münchener medizinische Wochenschrift*, 1904, lxxiv, p. 2038.

showed that the veins in the dorsum of the hand empty themselves immediately when the hand is lifted above the axilla. Hooker¹³ has found, however, by a modification of von Recklinghausen's method that the top of the column stands 2 to 16 cm., with an average on a number of persons of 9 cm., above the level of the tip of the sternum. During muscular exertion the top of the column¹⁴ rises above the clavicle so that the jugulars are visibly distended, and Hooker found pressures in some cases during vigorous exertion of 20 cm. or more above the tip of the sternum.

While the foregoing considerations tend to support the opinion that the venous supply and pressure are at all times during health sufficient to fill the right ventricle as rapidly as it relaxes, they indicate also that the pressure is not much more than the critical figure. It is probable that in sickness or in even a slight degree of physical weakness venous pressure may fall to a greater or less extent below normal, and especially when the subject assumes an erect position. The heart's tidal volume will then be considerably decreased.

As physiologists in the past have generally neglected venous pressure, it appears probable that a large part of all the investigations upon the circulation have been made under the abnormal conditions of subcritical venous pressure.

VII. SIGNIFICANCE OF THE RESULTS AND EVIDENCE AGAINST THEM.

Our observations confirm the earlier work from this laboratory, but so far as we can see are irreconcilable with the conceptions of the heart's behavior held by Zuntz,¹⁵ Plesch,¹⁶ Krogh,¹⁷ and their adherents. From calculations based upon the oxygen capacity of blood, the pulse rate, and the total oxygen consumption of the body, they have

¹³ HOOKER: this Journal, 1911, xxviii, p. 235.

¹⁴ What we speak of as the "top of the column" was called the "surface of the pre-ventricular reservoir" by VON RECKLINGHAUSEN: *Archiv für experimentelle Pathologie und Pharmakologie*, 1906, lv, p. 476, and 1907, lvi, p. 1.

¹⁵ ZUNTZ: *Zeitschrift für klinische Medizin*, 1912, lxxiv, nos. 3 and 4; also MARKOFF, MÜLLER, and ZUNTZ: *Zeitschrift für Balneologie*, 1911, iv, nos. 14 and 15.

¹⁶ PLESCH: *Zentralblatt für Physiologie*, 1912, xxvi, p. 89.

¹⁷ KROGH: *Skandinavisches Archiv für Physiologie*, 1912, xxvii, pp. 126 and 227; KROGH and LINDHARD: *Ibid.*, p. 100.

estimated that during physical exercise, when the heart rate is of course very rapid, the systolic discharge is a very large quantity, much larger than during bodily rest. Bohr,¹⁸ on the contrary, by ingenious measurements of the dead heart has attempted to show that the ventricle is incapable of so big a discharge, for he found that the pericardial sack is not large enough to allow the ventricle to hold such a volume. On this point Zuntz has attempted a refutation. Zuntz, Plesch, and Krogh admit, however, that in order to discharge the volume which their calculations demand *the ventricle must relax and be distended almost to its utmost in diastole and contract nearly to complete emptiness during systole*. Our experiments demonstrate, on the contrary, that, even when the rate of beat is slow, *this is an act which the heart is incapable of performing*.

In our experiments the pericardial sack was opened, while in Bohr's method the cardiac capacity is measured with the sack intact. The capacity is of course considerably less under the latter condition than under the former, but even this volume is far more than the ventricles ever discharge at a single beat. The behavior of the heart is quite unlike that of the respiratory mechanism.¹⁹ The ventricles never pass in a single stroke from utmost fulness to extreme contraction. If, owing to a high or low tonus, one limit is approached, the stroke always falls considerably short of the other. Zuntz may be correct regarding the volume of the dead heart, but Bohr was nearer the truth in respect to the maximum stroke of the living ventricle.

An even greater difficulty in the way of such calculations of the systolic discharge as those recently published by Krogh is the fact that *they require the ventricle to make maximal strokes at rapid rates of beat*. Our experience without exception demonstrates, on the contrary, that *at rapid pulse rates the abbreviation of diastole renders the relaxations much less complete and makes the systolic discharges therefore much smaller than at slow rates*.

We frankly admit the strength of the argument of Zuntz and his supporters that unless the arterial blood stream during muscular exertion is four or five fold greater than during bodily rest it will be insufficient to transport all of the oxygen absorbed by the lungs. As a man's pulse increases from 65 or 70 per minute during rest up to 140

¹⁸ BOHR: Skandinavisches Archiv für Physiologie, 1909, **xxii**, p. 221.

¹⁹ Cf. PLESCH: *Loc. cit.*

or 160 during hard work, the volume of the strokes of the heart must also be doubled in order to produce such an acceleration of the current as they believe to occur. If, on the contrary, the amplitude of stroke is progressively less as the rate of beat increases, as our observations indicate, the utmost acceleration of which the circulation is capable is less than double the resting value. If the circulation in a normal man during hard work behaves in this manner, it appears that the blood stream cannot carry all of the oxygen which he consumes, and that *there must be a huge oxidation in the lungs of incomplete combustion products brought from the laboring muscles.*

As is well known, Bohr held tenaciously to the belief in an extensive consumption of oxygen in the lungs, or in the blood during its passage through the lungs. Owing, however, to the wide variations in the results obtained and the extremely abnormal conditions maintained in his experiments with Henriques,²⁰ the theory has gained few supporters. The evidence which our observations afford on this matter is indirect, and direct determinations are necessary in so important a matter. Unless, however, experimental procedures can be found which will induce the heart to behave in conformity to the requirements of the current theory of pulmonary inactivity, the presumption must be in Bohr's favor.

Fortunately the whole matter hangs upon the crucial question whether the ventricles are smaller during physical exercise than during rest, and it should be possible to decide this point by means of the X-ray. Extensive studies of the effects of exercise upon the volume of the heart by this method are on record. The results are in complete agreement with our plethysmographic studies and with the requirements of Bohr's theory of pulmonary oxidation. Zuntz has made the point, however, that all such observations have been taken after, instead of during, the period of physical exertion. He regards the high venous pressure produced by the contraction of the skeletal muscles as the essential factor in the production of the large tidal volumes of the heart which he believes to occur. He considers that this factor disappears within a few seconds after the exertion ceases, and before the X-ray observation is taken. To meet this objection, we plan to make observations upon a man working upon a stationary

²⁰ BOHR and HENRIQUES: *Archives de physiologie*, 1897, ix, p. 459.

bicycle, so that the size of the heart can be observed during vigorous exercise.

VIII. CONCLUSIONS.

At venous pressures below 50 mm. of saline the amplitude of the heart's strokes varies with the pressure. At the critical figure of 50 mm. of saline and at all higher pressures the right ventricle is filled as rapidly as it relaxes, and the amplitude of stroke (*i. e.*, the volume of the systolic discharges) is maximal for the prevailing rate of beat.

The negative pressure maintained around the heart by the elasticity of the lungs is approximately equal to the critical venous pressure. If, as we hold, the pressure in the thoracic veins is normally equal to or slightly above atmospheric pressure, the heart under natural conditions is at all times supplied by an effective venous pressure (*i. e.*, the difference in pressure inside the veins and outside the heart) sufficient to maintain maximal efficiency for the prevailing rate of beat.

So long as the venous pressure is above the critical value the amplitude of beat is determined by the duration of diastole according to the principle of the superimposability of the volume curve. The rate of beat then determines the volume of the blood stream.

Extremely high tonus tends to render the ventricles more than normally resistant to distension. Low tonus tends to have the opposite effect.

In both hemorrhage and circulatory shock the decrease in the venous supply to the right heart is the critical factor. In this they differ from vasomotor failure in which the peripheral resistance of the arterial system is decreased.

Our observations are irreconcilable with the theory of Zuntz, Plesch, and Krogh, that the heart effects larger systolic discharges at the rapid pulse rates of physical exercise than at the slow rates of bodily rest. On the contrary, abbreviation of diastole necessarily decreases the amplitude of the heart's strokes.

This fact, coupled with the calculations of oxygen consumption in relation to the volume of the blood stream made by Zuntz and by

Krogh, lends strong, although indirect, support to Bohr's theory of a large pulmonary oxidation during vigorous muscular work.

We hope to be able to decide this question by means of X-ray observations on the heart during exercise. We shall shortly publish also some studies upon the influence of respiration on the circulation.

THE DEVELOPMENT OF DOUBLE REFRACTION IN THE MUSCLES OF FISH EMBRYOS.

By FREDERICK W. ELLIS.

SINCE the discovery of the double refraction of muscle by Boeck in 1839 physiologists and histologists have devoted much time to the study of the action of contractile tissues on polarized light. Brücke made an elaborate investigation of the anisotropy of striated muscle in 1857, but it is to Engelmann¹ that we owe the most extensive researches upon the double refraction of various cells and tissues. This eminent physiologist ascribed much importance to the double refraction of contractile tissues. He summed up his views upon the subject in the statement that wherever and in whatever form contractility appears, it is always due to the presence of doubly refracting, positive, uniaxial particles whose optical axes coincide with the direction of shortening. He believed that the double refraction and the contractility are due to minute morphological elements which he called inotagmata. His theory of muscular contraction, of which he gave an exposition in his Croonian lecture,² is based upon the action of heat upon these inotagmata, or their turgescence from their absorption of liquids.

The coincidence of contractility and double refraction is so very frequent that the question if it is universal is interesting and important. Engelmann admitted that structureless protoplasm, like that of *amœba*, does not exhibit any double refraction. He believed, however, that his inotagmata are present here, but that they are not arranged in an orderly manner, and, consequently, that their double refraction is masked. Most physiologists will probably prefer to

¹ ENGELMANN: *Archiv für die gesammte Physiologie*, 1875, xi, p. 432; also *Sitzungsberichte der königlichen preussischen Akademie der Wissenschaften*, 78th October, 1906.

² *Nature*, March 28, 1895.

assume that a double refraction that cannot be detected is non-existent.

Anisotropy is so common in inorganic as well as organic nature, and occurs under such a diversity of circumstances, that those who ascribe to it any especial significance in the explanation of physiological processes should clearly prove their contention. Peculiarities of molecular arrangement of a certain kind give rise to double refraction. Vegetable fibres and the cellulose framework of plants have nearly as marked an effect on polarized light as anisotropic crystals. We should expect that tissues of a fibrous character, or those having elements regularly arranged in longitudinal series, would exhibit doubly refractive properties. This is pre-eminently the case with striated muscle, which of all animal tissues is most markedly anisotropic. If it can be proven that muscle, at a certain stage in its development, possesses contractility without exhibiting double refraction, it cannot be claimed that double refraction is essential to muscular activity, or is anything more than a morphological accident.

G. Valentin^{*} stated that the heart of the chick embryo shows no signs of double refraction for the first six days, although it begins to beat on the second day. Engelmann contradicted this assertion with the statement that double refraction is present in the last half of the second day, when contractions begin. He stated that the double refraction is slight at this time, and that, in order to detect it, it is necessary to have the head screened from extraneous light with a "Dunkelkasten."

According to Engelmann the muscles of the tail and body of larval frogs exhibit double refraction as soon as contractions are recognizable. He makes the additional statement that double refraction and cross striation usually appear simultaneously, but that the striation may be delayed. As he expressed it, double refraction and not striation is the indication of the ability to contract.

The observations of Valentin and of Engelmann regarding the embryonic development of double refraction are all of which I have found any record. It is this line of investigation which promises most in determining the physiological significance of anisotropy.

The development of the embryos of certain species of fish having

^{*} VALENTIN, G.: Die Untersuchung der Pflanzen- und der Thiergewebe im polarisirten Lichte, Berlin, 1861.

transparent eggs offers most excellent opportunities for studies of this character. The eggs of the yellow perch furnish especially valuable material for this purpose. They are particularly well adapted for continuous observation. Both the eggs and the embryos are very transparent, consequently details of structure can be easily studied with the microscope before and after the hatching of the embryos. In the later stages of development the circulation of the blood is clearly seen in the entire animal. The eggs are hardy, and can easily be preserved in a living condition in the laboratory. The ease with which they can be found at the proper season also renders them valuable as objects of study. The eggs are surrounded by a thick gelatinous coating, and adhere to one another in ribbons or sheets. As these are deposited in shallow water on the borders of the ponds or lakes in which the fish are found, they are discovered with little difficulty.

In the spring of 1912 I studied the development of the yellow perch with the polarizing microscope. The first lot of eggs was taken from a pond April 17. The spawn had probably been deposited and fertilized within twenty-four or, at the most, thirty-six hours. The eggs were kept in a large dish in the laboratory, and the water was changed at frequent intervals. To examine the eggs a small portion of the sheet was cut off and slightly compressed between two round glass plates fastened in the ends of short pieces of brass tube. The pieces were of such a size that one slid with friction within the other. This was found to be a very convenient arrangement, as any degree of pressure could be applied, and the tubes could be rotated with the fingers on the stage of the microscope. The polarizing apparatus was fitted to a Zeiss stand with an Abbe condenser. A plate of selenite could be placed between the polarizing prism and the condenser. The object was viewed with a 17 mm. or a 12 mm. objective.

At the time of the removal of the eggs from the pond a germinal disk had formed. Development proceeded rapidly on the following day. The next day, April 19, the oval anlage of the eye appeared. The eye was distinctly marked off on April 20, and the tail began to project beyond the yolk sac. The notochord and the divisions of the brain were also apparent. Upon April 21 there were spontaneous movements of the tail, which was largely developed. The heart was beating regularly and slowly. The auditory sac was first

seen upon this day. No double refraction was detected in any part of the embryo. The first evidence of double refraction appeared the next day, the fifth from the beginning of the observations. There were slight indications of anisotropy in the muscles on each side of the notochord, most marked in the middle third of the body. The affected muscles had a silver-gray lustre when the nicols were crossed and the longitudinal axis of the embryo formed angles with the polarizing planes. When a film of selenite showing a purple transition tint in polarized light was placed above the polarizing prism with its axes forming angles of 45 degrees with the polarizing planes of the crossed nicols, blue or yellow complementary colors appeared in the anisotropic muscles, when the object was rotated. Two minute, doubly refracting spots, the beginnings of the otoliths, appeared in each of the auditory sacs upon this day. The embryo was about 4 mm. in length. April 24, double refraction was well marked in the longitudinal muscles of the body. The embryo was $5\frac{1}{2}$ mm. long. The eyes were pigmented, and blood corpuscles were seen circulating in the heart and blood vessels. With a selenite film of the first or second order the microscopic picture was very beautiful. The tiny otoliths shone like stars. The long blue or yellow band of muscle on each side of the notochord was a brilliant contrast to the purple ground. The rest of the embryo was isotropic. The heart showed no signs of double refraction at this time, and remained free from it for many days afterwards.

The embryos were kept under observation for more than two weeks longer. The double refraction increased in brilliancy for several days, and was confined at first to the muscles lying on each side of the notochord. At a later date thin muscles showing the phenomenon appeared in the pectoral fins and in the region of the lower jaw.

As a control experiment, another lot of eggs was obtained, April 25, from the same locality as the first. Owing to the difference in the temperatures of the pond and the laboratory, the No. 2 embryos had not developed as rapidly as those kept in the laboratory. Their stage of development corresponded fairly well with that of the No. 1 embryos on April 20. They were kept in the laboratory at the same temperature as the No. 1 eggs. On April 27 sluggish movements of the body and heart were first noticed, but no double refraction could

be detected. Slight double refraction of the body muscles appeared on the following day, but the heart was isotropic.

As a further control, another lot of eggs (No. 3), showing rather more development than No. 2, was taken from the pond in the afternoon of April 25. The embryos showed sluggish movements of the tail and heart; but, even on the following day, no double refraction of the muscles could be found. On April 27 beginning double refraction of the body muscles was detected.

The evidence afforded by the heart of the perch embryo that double refraction is not necessary for functional activity is very striking and complete. Some of the eggs of the three lots mentioned were kept under observation until the middle of May, but at no time was I able to convince myself that the heart showed any double refraction. Up to May 1 there was nothing to interfere with a clear and complete view of the heart, which was always isotropic. May 3 it was noted that the small thin muscles which move the lower jaw and are located near the heart were beginning to exhibit double refraction. As these muscles became more developed and more strongly anisotropic, there was increasing difficulty in determining whether the heart was isotropic; but the portions of the heart which were not covered by these muscles were apparently free from double refraction. Although the embryos were under observation nearly a month, it was not possible to determine when double refraction of the heart muscle of the fish begins, and a longer observation would probably have been unsuccessful owing to the growing opacity of the surrounding tissues, and the development of various overlying muscles.

In addition to the eggs mentioned three more portions were taken from the pond at various times. No. 4 eggs were removed May 4; they showed the commencement of double refraction in the body muscles. No. 5 eggs were secured May 7; the embryos were vigorous and lively. The No. 6 embryos were taken May 13, and had escaped from their envelopes. At no time was there unequivocal evidence of double refraction in the heart muscle of any of the embryos examined.

This series of observations seems to prove that double refraction is not a necessary accompaniment of contractility. Any theory of muscular contraction based upon the double refraction of

muscular fibres cannot be tenable. The interval of time between the appearance of spontaneous muscular contractions and the first indications of double refraction in the fish embryos was about a day. Development takes place so rapidly in the first five or six days after fertilization that a day, or even a few hours, is a relatively considerable portion of time.

The most convincing evidence that double refraction is not essential to muscular contraction was furnished by the embryonic heart. The circulation was carried on vigorously for days, and even weeks, without any appearance of double refraction in the heart muscle. The heart of the adult fish is anisotropic, but I was unable to determine in this research when the double refraction begins. It may be objected that it is possible that a slight degree of anisotropy was present and escaped detection. As the walls of the embryonic heart are thin, it was not to be expected that the action of its tissue on polarized light would be at all comparable to that of the thick masses of skeletal muscle, but some indubitable evidence of double refraction should have been obtained if we are to base a theory of muscular contractility on its presence. After the appearance of double refraction in the voluntary muscles it was easy to demonstrate, by crushing the object under a cover glass, that a single muscular fibre showed the phenomenon clearly. The thin muscles which appeared in the later stages of development in the region of the heart, the thickness of which probably did not exceed that of the walls of the heart, were unmistakably anisotropic. I did not use a "Dunkelkasten," but some of the later observations were made in a darkened room, extraneous light being excluded almost completely from the stage of the microscope. Even if it were true that a minimal amount of double refraction was present in the heart and was not detected, the contention that double refraction has anything to do with contractility would not be proven. It would be reasonable to expect, if Engelmann's theory is correct, that a heart that has been beating regularly and vigorously for days and even weeks would show decided evidence of anisotropy. In the absence of such evidence we are not justified in regarding double refraction as having anything more than morphological significance.

THE DEPRESSOR EFFECT OF ADRENALIN ON ARTERIAL PRESSURE.

By W. B. CANNON AND HENRY LYMAN.

[From the Laboratory of Physiology in the Harvard Medical School.]

IN 1911, during an investigation of the effect of splanchnic stimulation on the fatigue of skeletal muscle,¹ a rise of blood pressure, followed by a fall and a later rise, was repeatedly noticed while the nerves were being excited (see Fig. 1, A). Since the object of the investigation was to determine whether secretion from the adrenal glands influenced muscular fatigue, elimination of the direct vascular effects of splanchnic stimulation became necessary. Accordingly the coeliac axis, the superior and inferior mesenteric and the renal arteries were tied, and the experiment was then repeated. Splanchnic stimulation now caused an almost or quite sheer fall of pressure (see Fig. 1, B). This fall was obtained repeatedly under the conditions above described; it occurred after all nervous connections with the aorta and inferior vena cava had been excluded, and furthermore it occurred after direct stimulation of one of the adrenal glands. It seemed, therefore, that in the cat, the animal used in these observations, a substance was produced by the adrenal glands which caused a pure fall of blood pressure.

PREVIOUS OBSERVATIONS OF A DEPRESSOR EFFECT OF ADRENALIN.

Scattered through the literature on the adrenal glands can be found references to a depressor effect on arterial tension produced by adrenal extract or adrenalin itself. Bardier and Fränkel in 1899 observed that in two instances injection of adrenal extract induced a vasodilation in kidneys and spleen as shown by oncometer regis-

¹ CANNON and NICE: this Journal, 1912, xxix, p. xxiv.

tration.² In 1900 Moore and Purington reported that extracts of adrenal medulla of the ox caused, when given in exceedingly minute doses, a fall of blood pressure in the dog. Larger doses resulted in the typical rise of pressure. They suggested that the extract might contain two substances, the depressor substance stronger because effective at greater dilution; but they also admitted that a single substance might be present having opposite effects according to its dosage.³ These observations have been variously explained away. PARI found in a few instances an increased flow through the perfused kidneys and hind legs of dogs when a weak adrenalin solution kept for some time was added to the circulating fluid, but this did not occur if the adrenalin solution was fresh. He expressed the belief, therefore, that adrenalin, even in very small doses, never causes (in rabbits) a fall of arterial pressure, and he suggested that the results obtained by Moore and Purington were due to chemical changes in the dilute extracts used by them.⁴ Vincent has intimated that in these and in other instances of a depressor constituent of the adrenal glands⁵ the common power of tissue extracts to lower blood pressure may be invoked in explanation.⁶ Thus doubt is thrown on

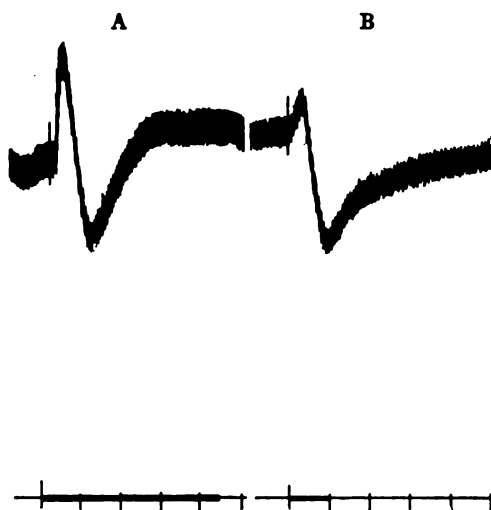


FIGURE 1. — *A*, blood-pressure changes during stimulation of left splanchnic nerves two and one-quarter minutes. *B*, the same, after tying arteries of the splanchnic area; stimulation, half a minute.

² BARDIER and FRÄNKEL: *Journal de physiologie et pathologie générale*, 1899, i, p. 960.

³ MOORE and PURINGTON: *Archiv für die gesammte Physiologie*, 1900, lxxxi, p. 483.

⁴ PARI: *Archives italiennes de biologie*, 1906, xlvi, p. 218.

⁵ See GÜRBER: *Sitzungsberichte der physikalisch-medizinischen Gesellschaft*, Würzburg, 1897, iv, p. 54; HUNT: *this Journal*, 1900, iii, p. xviii.

⁶ VINCENT: *Internal secretion and the ductless glands*, London, 1912, p. 174.

the validity of the inference that adrenalin in tissue extracts can lessen arterial tension.

Results concordant with those recorded by Moore and Purington, however, have been noted by persons who have employed not adrenal extracts, but pure adrenalin. Thus S. J. and C. Meltzer reported, within three years after Moore and Purington's observation, that subcutaneous injection of adrenalin caused dilation of the blood vessels of the rabbit's ear in normal conditions, but constriction if the nerves to the vessels were cut.⁷ In his summary of the action of adrenalin (1905), Elliott stated that he had seen extraordinarily dilute solutions of adrenalin cause a lowering of blood pressure in the cat, but that solutions of 1: 600,000 or 1: 200,000, if "slowly and exactly made," produce only rise of blood pressure.⁸ "Straight-forward experiment," Elliott declared, "fails to prove vascular dilation by adrenalin." By previously injecting active preparations of ergot, however, Dale showed that adrenalin could cause a marked fall, due to vasodilation. The discussion of this result will be deferred till later; for the present the fact need only be noted that, after injections of ergotoxine, adrenalin becomes a pure depressant, even in large doses.⁹ Further evidence that ergotoxine is not necessary for this effect was brought forward last year by Hoskins and McClure, who demonstrated that the fall of blood pressure in dogs, noted by Moore and Purington, was as true for minute doses of adrenalin as for minute doses of adrenal extract.¹⁰ And Elliott has recently examined the reason for failure to secure an even rise of pressure during excitation of the splanchnic nerves. He has noted that the disturbance (a drop) in the curve coincides with signs of adrenalin being freed in the body (dilation of the pupil), and has shown that in the absence of the adrenal glands the effect can be simulated by injecting adrenalin during splanchnic stimulation.¹¹ All these observations taken together indicate that adrenalin does

⁷ MELTZER, S. J. and C: this Journal, 1903, ix, p. 261.

⁸ ELLIOTT (Journal of physiology, 1905, xxxii, p. 411) did not mention the dosage or the rate of injection when these solutions raised the pressure, and both are important factors.

⁹ DALE: Journal of physiology, 1905, xxxii, p. lix; 1906, xxxiv, p. 169.

¹⁰ HOSKINS and MCCLURE: Archives of internal medicine, 1912, x, p. 353.

¹¹ ELLIOTT: Journal of physiology, 1912, xliv, p. 405.

not always increase arterial tension, but, in small doses, may indeed lower the tension. The almost direct fall in the blood pressure record reproduced in Fig. 1, *B* is therefore due to the liberation of adrenalin from the adrenal glands when the splanchnic nerves are stimulated.

In spite of the foregoing evidence the idea is firmly fixed that adrenalin is normally an agent raising blood pressure by vaso-constriction. For example, Biedl, in his summary of the literature on the ductless glands, describes, as the most important and most characteristic of the physiological effects of adrenalin, its action in raising blood pressure, and although mentioning the secondary fall observed in some animals states that the rise occurs even with minimal doses.¹² Vincent likewise has testified that although he has frequently employed very small doses, he has never seen the depressor effect of adrenal extract.¹³

THE OBJECTS AND METHODS OF THE PRESENT INVESTIGATION.

An investigation of the depressor action of adrenalin is important for several reasons. As already shown, the adrenal secretion poured out in the first moments of stimulation of splanchnic nerves causes blood pressure to fall. Depression due to adrenalin may therefore be a common phenomenon in conditions affecting these nerves, as strong sensory stimuli, pain, and major emotional states. Furthermore, the widespread conception that adrenalin is strictly vasopressor in action warrants a consideration of its depressor function. And, finally, because adrenalin mimics the sympathetic system, which is usually regarded as vasopressor, the vasodepressor action should be carefully considered. For these reasons the investigation here reported was undertaken.

Although Meltzer observed vasodilation of the rabbit's ear after a small subcutaneous injection of adrenalin, we have not been able to produce in the rabbit a fall of blood pressure with any dose, even the most attenuated. We have found no reference in the literature

¹² BIEDL: *Innere Sekretion*, Berlin and Vienna, 1913, second edition, i, pp. 429-430.

¹³ VINCENT: *Loc. cit.*, p. 174.

to a depressor action of adrenalin in the rabbit, and, according to Dale, the rabbit is exceptional in not showing a reversal of the pressor effect of adrenalin after ergotoxine. Hoskins and McClure showed that the dog might be used for the study. Because in the cat, however, the depressor action of splanchnic and adrenal stimulation was first seen by us, that animal was selected for the present investigation.

In order to produce uniform and readily repeated effects the adrenalin must be newly prepared, must not deteriorate during the course of the experiment, must be administered in carefully measured amount and at a carefully regulated rate. The preparation used was that made by Parke, Davis & Co., and was fresh. Immediately before the experiment it was diluted, usually 1:100,000, with distilled water, in which the adrenalin remains without chemical change much longer than in salt solutions. The injections were made into an external jugular vein, low in the neck, from a syringe with small barrel, graduated to tenths and fiftieths of a cubic centimetre. A sound which could be clearly heard recurring at intervals of one second permitted delivery of the dose uniformly in successive experiments. Thus it was possible to give .02 c.c. at the rate of .01 c.c. per second or 1 c.c. at the rate of 0.1 c.c. per second, or any intermediate combination of amount and rate of injection that was desired, and this could be repeated with satisfactory accuracy.

Usually the animals were anæsthetized with urethane. Since there is some relation between urethane anæsthesia and the efficacy of adrenalin,¹⁴ ether was used in some instances, and in others the cerebrum was destroyed during brief ether anæsthesia and the tests then tried on the decerebrate animal. The means of securing anæsthesia did not alter the effects of adrenalin, however, and consequently urethane (2 gm. per kilo, by stomach) was selected, both for its convenience and for its even action.

Blood pressure was registered by means of a mercury manometer connected with either a femoral or a carotid artery. In many instances the abdominal aorta, the subclavians and carotids were tied, limiting the circulation largely to thorax and abdomen. The pressure was then registered from one of the carotids. In all in-

¹⁴ See UNDERHILL: *Journal of biological chemistry*, 1911, ix, p. 1.

stances both vagus nerves were severed. An electric heating pad placed under the animal permitted the maintenance of normal temperature.

CONDITIONS FOR THE DEPRESSOR EFFECT OF ADRENALIN.

As shown in Fig. 2, a series of doses, varying from .3 c.c. to .05 c.c., of an adrenalin solution 1:100,000, injected each at the rate of



FIGURE 2. — Graded drops in blood pressure, with graded doses of adrenalin, 1: 100,000 (.3, .2, .1, and .05 c.c.) given at the rate of .01 c.c. per second. Time, half-minutes.

.01 c.c. per second, results in a series of drops in blood pressure, graded in accordance with the dose. Much more than .3 c.c. can be given at the rate of .01 c.c. per second — several cubic centimetres, in fact — without causing anything but a depression in the curve. In this connection the conclusion of Hoskins and McClure¹⁵ that the adrenals do not ordinarily produce sufficient secretion to affect the sympathetic system is eminently justified, for even when extra secretion is provoked by splanchnic stimulation the amount liberated at first is of the order demonstrated in Figs. 1 and 2, and in the cat, at least, reduces blood pressure rather than raises it.

The same amount will have different effects according to the rate

¹⁵ HOSKINS and MCCLURE: *Loc. cit.*, pp. 352, 353.

of injection. Thus .5 c.c. (1:100,000) given slowly (.02 c.c. per second) results in a pure fall of pressure, and given more rapidly (.1 c.c. per second) results in a primary rise followed by a fall (see Fig. 7, last two doses). Similarly, increasing doses of a strong solution (*e. g.*, 1:10,000), injected at a uniform rate, evoke progressively changing effects — varying from a pure fall to a rise followed by an almost equal drop below the original level, and finally to an almost pure rise, with relatively little subsequent drop (see Fig. 3).¹⁶

Repeated doses of an amount which evokes a fall of pressure act additively to evoke a further fall. As shown in Fig. 4, however, the degree of effect soon reaches a maximum. After the pressure had fallen from approximately 160 mm. to 136 mm., further injections lowered it no more. The injection of .4 c.c. (1:100,000) in the same case — .1 c.c. at the rate of .01 c.c. per second, followed continuously by .1 c.c. at the rate of .02 c.c. per second, and that by .2 c.c. at the rate of .1 c.c. per second (see Fig. 5) — also reduced the pressure from 166 mm. to 136 mm. A similar result is seen in Fig. 2; .3 c.c. in that instance caused no further drop than .2 c.c., but the effect was more lasting. Of course, small doses do not lower the pressure

¹⁶ In a recent paper (Journal of industrial and engineering chemistry, 1912, iv, p. 636) WEIDLEIN, like PARI, has drawn the conclusion that the depressant property of epinephrin is due to impurities present in the gland and to decomposition products formed by oxidation. His evidence for this conclusion is, that with his repurified epinephrin, lowering of arterial pressure did not occur after the initial rise; whereas, with commercial or decomposed epinephrin, the initial rise was not so high and the subsequent depression was marked. WEIDLEIN used very strong doses — 1 c.c. of 1:10,000 in most instances, *i. e.*, from fifty to one hundred times the physiological doses used by us. It is quite possible that the depression became marked as his solutions deteriorated because the epinephrin injected in 1 c.c. was thus reduced to a more nearly physiological amount. We have in fact obtained a direct drop in blood pressure by use of the very pure ash-free epinephrin refined by TAVAU (see Bulletin No. 55, Hygienic Laboratory, U. S. Public Health Service, 1909, p. 23), which was kindly provided by Dr. REID HUNT. Furthermore, we have obtained a direct drop in blood pressure with .2 c.c. of fresh colorless commercial adrenalin (1:100,000), and then placed the solution in a warm bath in the sun for fifteen minutes, when it turned pink. Now the same dose (.2 c.c.) still caused a fall of blood pressure, but no greater fall than before. The solution was again exposed to the sun for fifteen minutes and was changed to a much deeper pink. Now the same dose had no effect whatever upon blood pressure, and even when 5 c.c. were introduced failed to cause a drop. As the material decomposed, therefore, it became not depressant but inert.

as much as large doses, but, on the other hand, the depressive power is limited, and the limit may still leave the pressure at a high level.

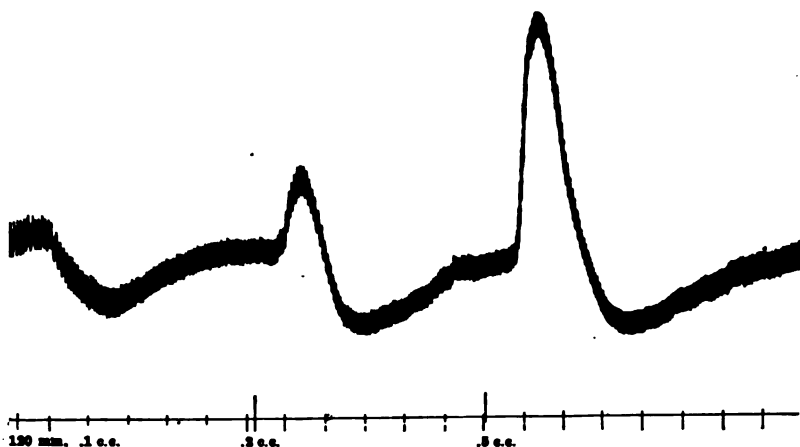


FIGURE 3.—Change from pure fall in blood pressure to fall and rise, and to predominant rise, with increasing doses (.1 c.c., .2 c.c., and .5 c.c.) of adrenalin 1:10,000. Time line, 120 mm. Hg; time, half-minutes.

The percentage fall has varied, for doses of .5 c.c. (1:100,000), between 13 and 22 per cent; and in a given case the percentages have been fairly constant at different initial pressures. Thus in three distinct cases the figures were as follows when a maximum straight drop¹⁷ resulted from a single dose:

Original pressure. mm.	Drop. mm.	Percentage drop.
150	34	22
136	30	22
162	34	21
116	26	22
160	24	15
150	20	13
162	24	15
146	20	14
164	32	19
180	34	18

¹⁷ Usually the drop produced by a dose that causes a slight rise at first, is greater than one not preceded by the rise. In one such case the drop amounted to 54 mm.

In one instance, after the subclavian and carotid arteries and the abdominal aorta below the kidneys had been tied, and the left adrenal gland excluded, arterial tension was registered at 80 mm. Now .1 c.c. adrenalin (1:100,000), injected .02 c.c. per second, lowered the tension 22 mm. The left splanchnic nerve was then stimulated and the tension thereby increased to 118 mm. The same dose as before, introduced during the stimulation, lowered the pressure 32 mm. In the first condition the percentage drop was 27.5, in the second 28. It appears, therefore, that the ratio of fall to initial pressure is fairly constant in a given case, and with arterial tensions of medium value.

If the arterial tension is lowered, however, by compression of the heart through the thoracic wall, or by placing clips on the portal and inferior caval veins, the percentages vary widely from what is average under more normal conditions. The pressure may be thus lowered mechanically to 70 or 80 mm. of mercury: A dose of adrenalin (.5 c.c., 1:100,000) then causes the pressure to fall still further — in one case 10–12 mm., *i. e.*, about 15 per cent. In this animal the pressures ranged normally between 116 and 162, and the percentage fall, with the same dose, was about 22 (see first group of figures, p. 383).

THE DEPRESSOR EFFECT OF ADRENALIN DUE TO VASODILATION.

Examination of the arterial pressure curve registered on a rapid drum reveals no noteworthy alteration of the heart rate, and only slight diminution of the pulse pressure as the drop occurs. A straight drop of 42 mm. in 38 seconds was produced by injecting .1 c.c. adrenalin (1:100,000) in 1 second, and even with this marked and rapid fall the heart showed no change of beat. The fall, therefore, probably results from lessened peripheral resistance due to vasodilation. This inference we have justified by showing that an increase in the volume of a hind-leg occurs at the same time with the lowering of blood pressure. The inference is also supported by Dale's observation that the depressor effect of adrenalin after ergotoxine is accompanied by increased volume of parts.

Because of evidence of vasodilator nerves in the head and limbs the question arose as to whether the depressor effect resulted from

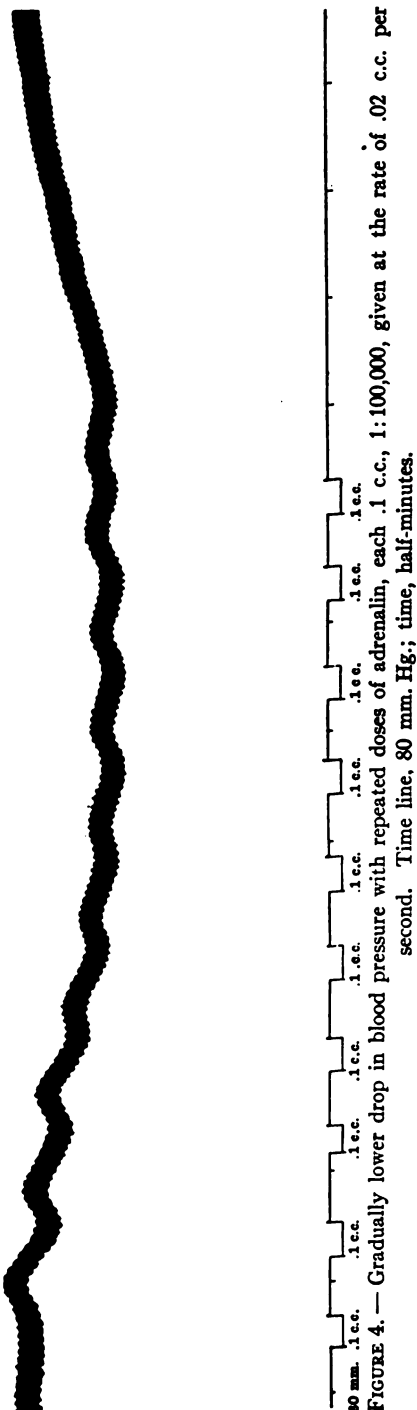
vasodilation exclusively in these parts of the body. Accordingly, after tying the internal iliac arteries, clips were placed on both subclavians, both carotids and the left iliac (the pressure was being registered from the right femoral). The pressure rose from 154 mm. to 190 mm. A dose of adrenalin (.5 c.c., 1:100,000) now caused a fall of 12 mm. The clips were removed and the pressure fell to 146 mm. The same dose now lowered the pressure by 22 mm. Next the inferior mesenteric artery was tied, and clips were placed on the superior mesenteric and renal arteries and on the coeliac axis. The pressure rose to 166 mm., and the dose, again repeated, caused a fall of 18 mm. Thus the vasodilation is not confined to any one field of the body, but affects alike the outlying limbs and the splanchnic area.

The character of the drop in pressure suggests that the adrenalin may affect the vasoconstrictor centre as the depressor nerve affects it — thus being able to replace impulses at one point in the central nervous system just as it may replace them in organs supplied by sympathetic fibres. In one instance only in our series did adrenalin wholly fail to induce a pure fall of pressure; by good fortune the depressor nerve was separate in this animal, and when stimulated induced a marked drop in the curve. Adrenalin probably does not, therefore, act like impulses delivered by the depressor nerve.

CONDITIONS CHANGING THE DEPRESSOR INTO A PRESSOR EFFECT.

As already stated, weak solutions of adrenalin injected more and more rapidly, or stronger solutions injected at a uniform rate but in gradually larger doses, are followed progressively first by a fall of pressure, then by a rise and fall, and finally by almost a pure rise. Thus the amount introduced in a given time is capable of changing the depressor into a pressor effect. But other conditions can be demonstrated which are favorable to this reversal, with no change in the rate of injection.

1. **Reversal after pithing.** — Pithing the brain and upper spinal cord was performed through an opening in the orbit. After this operation the blood pressure usually falls to about 50 mm. of mercury. As the fall is occurring, and immediately after a level is reached, the



adrenalin dose which was effective before has, in some of our cases, failed to produce any change. This insensitiveness, when present, soon disappears, and then the dose which previous to the pithing caused a pure drop in the curve now causes a pure rise (see Fig. 6, *A*, *z*, before pithing; and *B*, after pithing) — there has been an exact reversal of results.

Observations made before and after pithing show that the latent period, measured between the beginning of the injection and the beginning of the fall or rise, is approximately the same for the two effects. On one occasion, however, the rise appeared after seven seconds, whereas the fall was never seen in less than ten seconds after the adrenalin began to be introduced.

The rise after pithing is not due to any changed action on the heart, for a record made on a rapid drum shows no alteration of the rate of beat adequate to explain the new effect. The elevation of pressure, therefore, results from increased peripheral resistance — pithing has transformed the vasodilator action of adrenalin to a vasoconstrictor action.

The fact that adrenalin alone may thus have opposite effects rules out the first of Moore and Purington's suggestions, namely, that there might be two substances acting oppositely — one to reduce,

the other to raise blood pressure. The further fact here recorded rules out the necessity of their second suggestion (a single substance opposite in action according to dosage), for opposite effects are observed with the same dosage. The problem is thus reduced to a question of what reverses the effect of uniform doses of adrenalin. Before considering that question further, other conditions leading to reversal will be described.

2. *Reversal during depressor stimulation.* — If the depressor nerve is stimulated and adrenalin is then administered, the pressure can

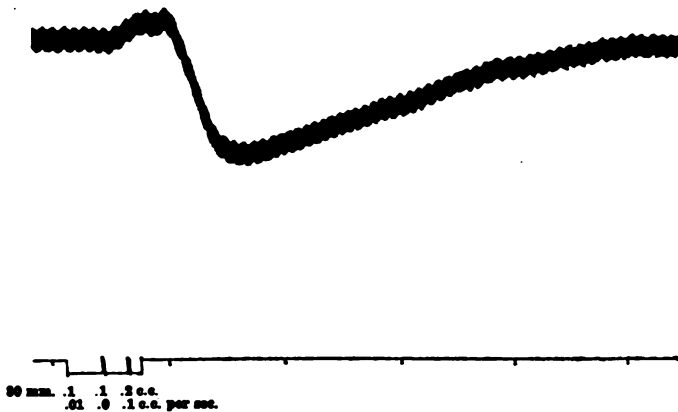


FIGURE 5. — Same case as Fig. 4. Drop in blood pressure after adrenalin, 1:100,000; .1 c.c. at .01 c.c. per second, then .1 c.c. at .02 c.c. per second, then .2 c.c. at .1 c.c. per second.

be made to fall further than if the depressor alone is stimulated. In Fig. 6, *A* is presented a record, taken from an animal with limb and carotid arteries tied, in which depressor stimulation alone caused a drop of 34 mm. Two doses of adrenalin (.2 c.c., 1:100,000) injected at the rate of .02 c.c. per second were given one after the other, while the stimulation of the depressor continued (to *y*), and the pressure was let down 40 mm. further. A third dose produced a brief rise followed by a drop of 10 mm. more. The original pressure of 184 mm. was thus reduced to 100 mm. Repetitions of the same dose of adrenalin now caused, not a further fall, but *elevations* of pressure; and when the pressure was restored to the original level the dose again caused a fall. In other words, without pithing or any other disturbance of the central nervous system than that produced

by stimulation of the depressor nerve, the effect of a small dose of adrenalin can be precisely reversed.¹⁸

3. *Effect of nitrites on the reversal.* — The ability of nitrites to lower blood pressure by action on peripheral structures is well known. If a small dose of nitroglycerine is given intravenously, the pressure falls, and then a dose of adrenalin that ordinarily produces a considerable reduction has no effect. As soon, however, as the change wrought by the nitrites is nearly past, and the former pressure is being recovered, the adrenalin again is able to exert a depressor action (see Fig. 7). After pithing, when all doses of adrenalin, even the minutest, cause an elevation of blood pressure, nitroglycerine is still capable of lowering it. Once when, after pithing, the pressure was only 26 mm., nitroglycerine caused a clearly marked further fall, and the least effective dose of adrenalin resulted in a rise. The failure of adrenalin to lower pressure after pithing is, therefore, not because the limits of vasodilation have been reached.

4. *Reversing the reversal by means of ergotoxine.* — As already stated, Dale has proved that, after pithing the brain and giving ergotoxine, even large doses of adrenalin cause a fall of pressure. The depres-

¹⁸ In this instance the failure of the pressure to rise after the first and second doses of adrenalin, as it rose again at *z*, may be regarded as due to absence of vasoconstrictor impulses, because of continued stimulation of the depressor nerve. When the tone had been further lost, however, after the third dose, the action of adrenalin became reversed. Evidently the tone may be considerably reduced without reversal, and yet a critical point is reached at which reversal occurs.

The oscillations following adrenalin dosage (see Fig. 6, *A*) have often been noted; they may indicate the operation of opposite factors working on the vascular wall and tending towards equilibrium.

In a few instances reversal has been observed during the high pressure that prevails for a short period after the brain is pithed. The brief sustained tone of the blood vessels under these circumstances may be due to temporary excitation of spinal centres by the trauma above, for the fall caused by adrenalin is followed by a rise. As the general blood pressure gradually drops, a level is soon reached, however, at which adrenalin evokes a rise instead of a fall.

On warming an animal to 44° C. the pressure fell from 150 mm. to 100 mm., and the adrenalin dose that produced a fall at normal temperature then produced a rise. Possibly in this case the result should not be attributed primarily to the effect of heat on the smooth muscle of the vessel walls, but to injury of the central nervous system and consequent loss of vascular tone.

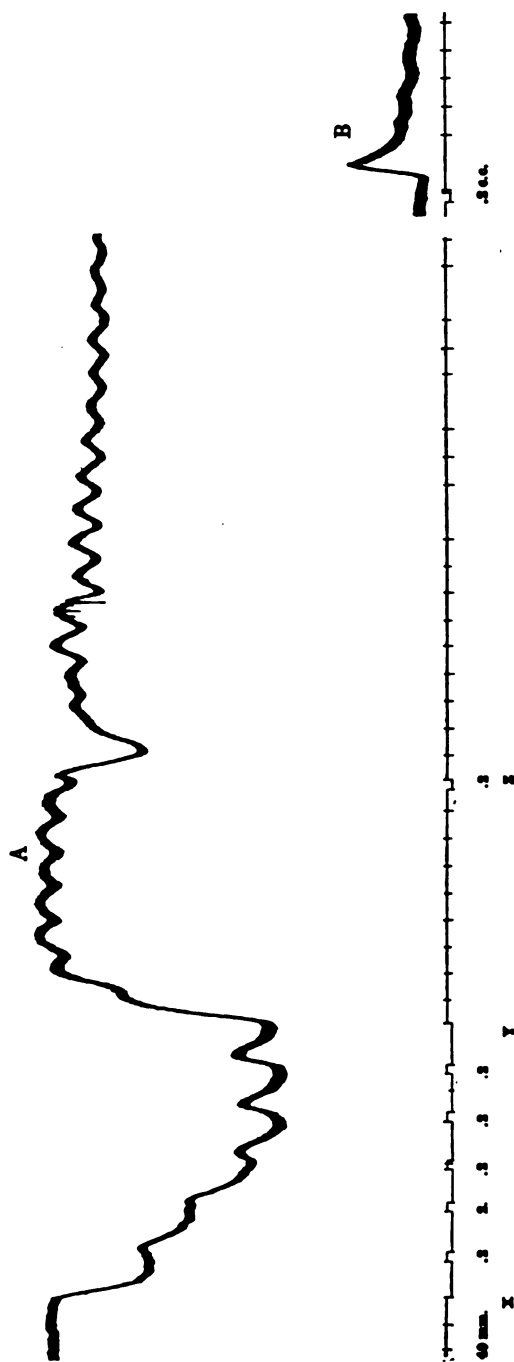


FIGURE 6.—Seven tenths the original size. A, successive drops in blood pressure during depressor stimulation (x to y) and with first three doses of adrenalin (1:100,000), then reversal of effect with last two doses. After pressure restored pure drop in pressure with same dose. Time line, 40 mm. B, same animal, after pithing down to mid-thorax. Rise of pressure with same dose as before. Time line, 40 mm. In every instance the dose was .2 c.c., given .02 c.c. per second.

sive action of adrenalin lost after pithing can be restored by ergotoxine with no change of dose.

In one case (that described on p. 387 and represented in Fig. 6), while the pressure was at 104 mm., .2 c.c. of adrenalin (1:100,000), given at the rate of .02 c.c. per second, caused a fall of 34 mm. (Fig. 6, A, 2). The brain, and the cord as far as the mid-thorax, were pithed,

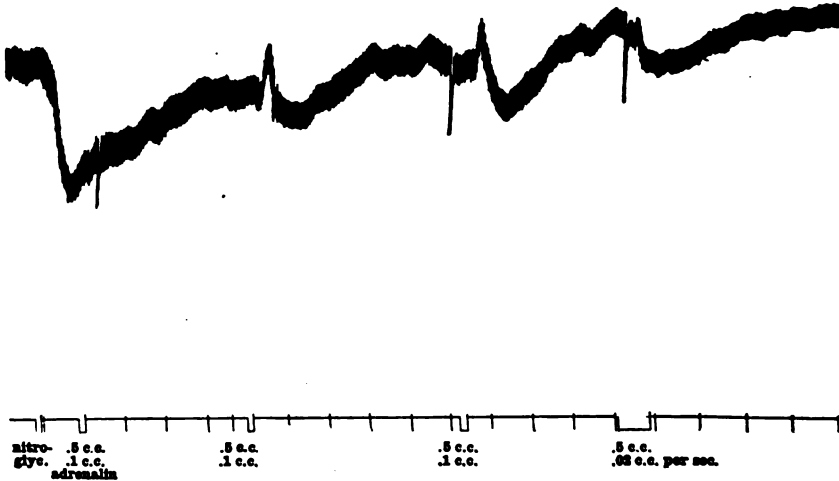


FIGURE 7. — First dose, .65 mgm. nitroglycerine. No fall from first dose of adrenalin given during nitroglycerine depression. Rise and fall after second and third doses adrenalin (1:100,000), .5 c.c., .1 c.c. per second. Pure fall after fourth dose, .5 c.c., .02 c.c. per second. Time, half-minutes.

and then, with the pressure at 50 mm., the same dose produced a rise of 26 mm. (see Fig. 6, B). The entire cord was now pithed and yet, by means of repeated doses of ergotoxine (.65 mgm. in each), the pressure was increased to 132 mm. Stimulation of the left splanchnic nerves, and injection of the same dose of adrenalin as before, each resulted in a marked fall of pressure. Three minutes later splanchnic stimulation caused, after a slight fall, a rise of 22 mm.; and when, during continuance of the stimulation, the same dose of adrenalin was repeated, a straight drop of 24 mm. occurred, and later 14 mm. more (see Fig. 8).

Evidently in the foregoing instance, in the complete absence of the central nervous system, the ability of a small dose of adrenalin to lower blood pressure was quite restored by giving ergotoxine.

This restoration is not the result of merely increasing the pressure. When that is done (after the pressure has once fallen in a pithed

animal), either by further pithing of the upper cord, or by injecting stronger solutions of adrenalin than those used in this investigation, or by stimulating the splanchnic nerves (after limb and neck vessels are tied), pressures from 140 mm. to 180 mm. can be temporarily produced. But doses of adrenalin which usually lower the pressure either have no effect under these circumstances or raise the pressure still higher.

DISCUSSION OF THE OPPOSED ACTIONS OF ADRENALIN.

Evidence against a central source of the depression. — Because the vascular depression from adrenalin is lost after destruction of the bulb and upper cord, it is natural to infer that the depression in the intact animal must be of central origin. The muscles of the arterial wall might thus be affected by two influences — depressive or negative influences, because of the action of adrenalin on the central nervous system; and stimulative influences, because of its action peripherally. And since depression is produced by doses weaker than those producing augmentation, the central nervous control would be proved more sensitive than the peripheral structures to adrenalin.¹⁹

Adrenalin might indeed act centrally to cause vasodilation, by stimulating the known vasodilators to the limbs²⁰ and head, but this possibility is rendered improbable by continuance of a marked depressor effect after all limb and head arteries are tied (see p. 385). Or adrenalin might lower pressure by inhibiting the tone of the vasomotor centre; but when that centre is in a tonic state and capable of being inhibited, as shown by action of the depressor nerve, adrenalin may fail to have any effect whatever (see p. 385). The assumption that the fall of blood pressure is of central origin meets another difficulty in accounting for the marked drop in pressure after ergotoxine, in an animal with the entire cord wholly functionless from pithing (see Fig. 8, *A*). The central nervous system, therefore, is not needed to explain vasodilation after adrenalin, and the evidence does not support the view that vasodilation is normally of central origin.

¹⁹ See MELTZER, S. J. and C.: *Loc. cit.*, p. 259.

²⁰ See BAYLISS: *Journal of physiology*, 1902, xxviii, p. 298.

Evidence against depression being due to blocking of vasoconstrictor impulses by adrenalin. — While arteries are connected with the central nervous system they are continually receiving impulses, which hold their muscular walls in tonic contraction against the internal pressure. Vasodilation under these circumstances might be due to

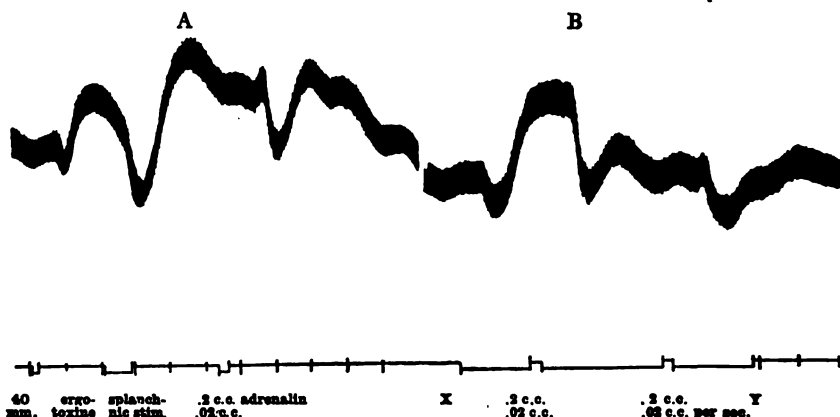


FIGURE 8. — A continuation of Fig. 6; blood pressure raised by ergotoxine. *A*, first rise from .65 mgm. ergotoxine; first fall from stimulation of left splanchnic nerves; second fall from .2 c.c. adrenalin (1:100,000), .02 c.c. per second. *B* (three minutes later), rise from splanchnic stimulation (*x* to *y*); two doses of adrenalin, each .2 c.c., .02 c.c. per second, cause fall. Time line, 40 mm. Hg; time, half-minutes.

a blocking of vasoconstrictor impulses. Thus, indeed, adrenalin might act. And certainly while pressure is high from stimulation of splanchnic nerves (in an animal with limb and neck vessels tied), the drop resulting from a small dose of adrenalin (see p. 384) strongly gives the impression that the splanchnic impulses are being blocked. But if that is the action of adrenalin, why does it not produce the same result when splanchnic nerves are stimulated after pithing or the pressure is raised by further destruction of the spinal cord? Under these circumstances the temporary rise is due to constrictor impulses delivered to the vessels, and yet, as already stated, adrenalin causes not a fall, but may cause an additional rise.

The drop of pressure from adrenalin after ergotoxine has been given to an animal with the cord entirely destroyed (see p. 390) is also inconsistent with the view that the drop results from the blocking of impulses, for the central nervous system has been abolished. This view, therefore, may properly be regarded as having little support.

Are the opposed effects of adrenalin due to stimulation of vasoconstrictor and vasodilator nerve endings? — If the depressor effect of adrenalin is not due to change in the central nervous system or to the blocking of vasomotor impulses from the spinal cord, the effect must be wrought in the muscle fibres themselves. This is the view which Dale has supported in accounting for vasodilation from adrenalin after ergotoxine had been administered. Ergotoxine, he explained, paralyzes the vasoconstrictor endings in the muscle, and adrenalin then reveals the action of the vasodilator endings which had formerly been overwhelmed and thus masked.²¹ We have had difficulty in accepting this explanation, for several reasons:

1. The evidence that the sympathetic system conveys vasodilator impulses is very meagre. Dastre and Morat described dilation of the vessels of the tongue when the cervical sympathetic was excited,²² but Elliott was unable to confirm the observation.²³ On stimulating the peripheral end of the right splanchnic nerve with break shocks at the rate of one in a second or one in two seconds, Bradford observed marked expansion of the kidney with either no change of blood pressure or a slight fall.²⁴ This result does not prove the distribution of peripheral dilators to the vessels of the splanchnic area in general, for the impulses that relaxed the kidney often did not equally affect the blood pressure. Also, from what is now known of the effects of adrenal secretion, the case of simultaneous dilation and drop in pressure might have resulted from liberated adrenalin — and this may have been a common source of error in other judgments of blood-pressure changes after splanchnic stimulation. Furthermore, even if vasodilator nerves are granted, Bradford's result does not prove that the dilation of the kidney was due to sympathetic impulses. Indeed Bayliss has explained instances of visceral dilation on the basis of his studies of dilation of limb vessels, as evoked by antidromic conduction in nerves of the dorsal roots.²⁵

2. The known and well-established vasodilator nerves (apart from the dorsal roots of spinal nerves) do not belong to the thoracico-lumbar

²¹ DALE: *Loc. cit.*, p. 201.

²² DASTRE and MORAT: *Comptes rendus, Académie des Sciences, Paris, 1880*, p. 393.

²³ ELLIOTT: *Journal of physiology*, 1905, xxxii, p. 411.

²⁴ BRADFORD: *Ibid.*, 1889, x, p. 387.

²⁵ BAYLISS: *Loc. cit.*, p. 280.

(sympathetic) division of the autonomic system, but to the cranial and sacral divisions. In vessels and in other structures where fibres from the middle division meet fibres from either of the terminal divisions, the two are set in opposition. The opposition, in other words, lies not within the sympathetic, but between the sympathetic and the upper or lower group of autonomic nerves. And the action on arterial walls which the sympathetic opposes to these nerves is invariably constriction.

3. Adrenalin, as Elliott has shown, acts as if it stimulated sympathetic endings, or the myoneural junctions between sympathetic neurones and smooth muscle fibres. If the fall of blood pressure after small doses of adrenalin is due to stimulation of sympathetic endings, there must be vasodilator nerves in the sympathetic; but, as already explained, no well-supported evidence for the existence of such nerves in the sympathetic has been brought forward, and the assumption of their existence there contravenes the strongly established theory of the organization of the autonomic system. Furthermore, if weak doses of adrenalin stimulate vasodilator and strong doses vasoconstrictor endings, weak stimuli applied to splanchnic nerves might be expected to cause vasodilation and strong stimuli the opposite. This, however, is not true. After restricting the circulation to thorax and abdomen and tying off the left adrenal gland, we stimulated the left splanchnic nerves with electrical stimuli of various rates per second, and of various strengths — down to and below the threshold for any change whatever — and never obtained any other result than increase of blood pressure. The nerve impulses did not act, therefore, as they should have acted if weak adrenalin stimulates vasodilator endings.

4. Since weak electrical stimulation fails to support the view that weak adrenalin acts on vasodilator endings, there remains the possible application of Dale's suggestion — that adrenalin lowers blood pressure after ergotoxine because the latter paralyzes vasoconstrictor endings and leaves dilator endings still sensitive. This explanation applied to the observations recorded in the present paper involves assuming that vasoconstrictor endings are less sensitive than dilator endings normally, that pithing somehow renders the constrictor endings as sensitive as the dilator endings were before and wholly paralyzes the dilator endings, and that when ergotoxine is given and the

vessels are constricted, the constrictor endings are paralyzed, and the dilator endings are rendered sensitive again. Although there appears to be no good reason for excluding the possibility of this sequence of profound changes, yet so many ill-supported assumptions are involved, including the existence of sympathetic vasodilators, that it has seemed to us highly unsatisfactory.

Because the evidence for vasodilator nerves in the sympathetic system is slight; because the existence of such nerves, opposed to vasoconstrictor nerves in the same system, would not harmonize with the known organization of the autonomic system; because the depressor action of weak adrenalin described in this paper cannot be duplicated by weak stimulation of nerves to blood vessels; and because the reversed action of adrenalin here described involves assumptions as to effects on hypothetical nerve endings that seem to us unwarranted — for these reasons we are inclined to reject the view that adrenalin in small amount or after ergotoxine stimulates vasodilator nerves.

Does adrenalin act oppositely according to the state of the muscle? — The evidence thus far adduced has led us, by exclusion, to look for an explanation of the reversed action of adrenalin in the state of the muscle, for, after all, the primary observation on which this investigation was based was vasodilation when adrenalin was administered to vessels in high tonic contraction, and vasoconstriction when in a relatively atonic state.

Observations that the same agent applied to smooth muscle may have quite different effects according to the condition of the muscle have not been rare. Lepine noted that sciatic stimulation resulted in vasodilation if a leg is cooled, and in vasoconstriction if it is warmed.²⁶ Stewart observed that stimulation of the sacral nerves resulted in relaxation if the bladder was contracted, and in contraction if the bladder was relaxed.²⁷ Dale found that adrenalin caused relaxation of the non-pregnant uterus, but contraction of the pregnant.²⁸ Hooker has recently reported that carbon dioxide when applied to smooth muscle of the intestine causes it to relax if it is contracted and rhythmic, and to contract if relaxed and arrhythmic.²⁹

²⁶ LEPINE: *Mémoires, Société de Biologie*, 1876, p. 26.

²⁷ STEWART: *this Journal*, 1899, ii, p. 192.

²⁸ DALE: *Loc. cit.*, p. 189.

²⁹ HOOKER: *this Journal*, 1912, *xxi*, p. 53.

Vasodilation, when vessels are still receiving tonic impulses from the central nervous system, or when, after denervation, they have been brought into sustained contraction by ergotoxine; and, on the other hand, *vasoconstriction*, after the vessels have been heated to 44° C., after they have lost their tonic contraction by being separated from the central nervous system, or have been largely deprived of tonic impulses by extreme action of the depressor nerve — these two effects of adrenalin we would place with the other foregoing examples of change in opposite directions, produced by a single agent, acting on smooth muscle under different conditions.³⁰

To turn a group of results thus into the murky realm of smooth-muscle physiology for explanation is, of course, giving no explanation at all. In the presence of so much obscurity we hesitate to speculate as to the manner in which the opposite actions of adrenalin occur, and yet an hypothesis may be entertained if it is made to keep its proper place. On these terms we suggest the following possible way of regarding the phenomena described in this paper:

Contraction and relaxation, while separable processes, are directly and intimately related. The contracted muscle is prepared for relaxation, the relaxed muscle is prepared for contraction. Indeed, as a muscle is contracting it may become the seat of changes which, being incomplete, are unstable, and on being completed cause relaxation. Thus, for example, the contractile process might be due to partial oxidation; and the relaxation process might result from the further or total oxidation of the partly oxidized materials. Both contraction and relaxation could then be accounted for by a single procedure — that of oxidizing certain material in the muscle.³¹

In this view sustained contraction, or tonus, might be due to continuous production of partly oxidized materials at such rate that in spite of continuous further combustion they are present in excess. Under these circumstances any agent that would favor combustion might have two effects: by increasing the incompletely burned

³⁰ DALE has dismissed this explanation of the reversal of effect of adrenalin on blood pressure after ergotoxine because vascular tone may be lowered by large doses of ergot, and yet adrenalin causes relaxation. As shown in Fig. 6, A, however, lowering of the tone doses not result in reversal unless a certain critical stage has been reached.

³¹ Cf. MEIGS: *Journal of experimental zoölogy*, 1912, xiii, p. 548.

materials it would increase contraction, by burning the excess of those materials it would cause relaxation. In a weak form the agent might affect the terminal phase, in a strong form it might affect in greater degree the initial phase.

In the state of total relaxation the material used for contraction may be regarded as being undisturbed. The only change that can occur first now, when the muscle is stimulated, is such partial combustion as will result in contraction. And in the absence of anything to continue the incomplete burning, the partial process will go on promptly to completion, and relaxation will attend it.

Without doing violence to the relation of adrenalin to the sympathetic innervation of blood vessels, it is possible by such a scheme to conceive the various shifting effects of adrenalin described in this paper. Adrenalin mimics the sympathetic system in starting the contractile process. The relaxation process, however, may be regarded as merely another stage of the same change, not different in kind; and we may suppose that under certain circumstances sympathetic impulses and their substitute, adrenalin, are capable of affecting that also. Thus the vasopressor effect of adrenalin, the vasodepressor effect, the drop after ergotoxine both when the splanchnic is stimulated and when adrenalin is given, and even the failure of adrenalin to cause a fall when in a pithed animal pressure has been raised by nervous stimulation or by strong adrenalin — can, we think, have a possible, though very hypothetical, explanation. But at the present stage of our knowledge the detailed application of this hypothesis to every phenomenon above reported as resulting from adrenalin dosage seems not worth while.

SUMMARY.

Stimulation of an adrenal gland, or splanchnic stimulation after excluding splanchnic vessels, results in the cat in a fall of blood pressure due to vasodilation.

Injection of small doses of adrenalin (*e. g.*, .1 to .5 c.c, 1 : 100,000) at a uniform slow rate (*e. g.*, .02 c.c. per second), into a cat, causes a similar fall of blood pressure. This effect varies, within limits, with the dose and with the rate of injection. Repeated doses

causing vasodilation have a cumulative effect. The percentage drop in a given case is usually constant for the same dose, given at different initial pressures.

The depressor effect of adrenalin is changed into a pressor effect after pithing and after extreme depression from depressor stimulation combined with repeated relaxing doses of adrenalin. During the drop in pressure after pithing the brain the action of adrenalin may reverse from depressor to pressor. It reverses with high temperature (44°C.).

The depressor effect does not occur if arterial tension has been much lowered by nitroglycerine.

The reversal from depressor to pressor action after pithing can be again reversed to depressor action by ergotoxine (Dale).

Reasons are given for inferring that the depressor effect is not of central origin, is not due to blocking of vasoconstrictor impulses, or to stimulation of supposed vasodilator sympathetic endings by adrenalin.

The two effects, vasodilation and vasoconstriction, are attributed to opposite actions of adrenalin according to the state of the muscle — relaxation when tonically shortened, contraction when relaxed. Other instances of like character are cited.

A tentative hypothesis is offered to account for the opposite actions.

THE INFLUENCE OF RESPIRATION UPON THE VELOCITY OF THE BLOOD STREAM.

BY YANDELL HENDERSON AND THEODORE B. BARRINGER, JR.

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IT is generally believed that the movements of respiration afford considerable assistance to the heart in propelling the blood stream. The acceleration of the circulation by this means is supposed to be especially important during and after muscular exertion. According to the conception held by Zuntz, Plesch, Krogh, and their collaborators, the hyperpnœa of an athlete during and after exertion is an important auxiliary, or even a controlling factor, in producing those enormous systolic discharges in rapid succession which they believe to occur when the oxygen requirements of the body are at a maximum.¹

In previous papers we have brought forward evidence against this general conception of the heart's behavior. We have shown that even when the heart is beating slowly it is incapable of making strokes of the size which the theory of Zuntz and his adherents demands. The ventricles never pass from almost complete relaxation and fulness to nearly complete contraction and emptiness in a single stroke. We have demonstrated also that at such rapid rates of beat as occur during muscular exertion the diastoles are abbreviated and the strokes are therefore smaller than at slow rates. The volume of the arterial blood stream in unit time, *i. e.*, the output per second of the left ventricle, is determined under all normal conditions by the rate of beat acting upon the functional capacity of the individual heart as expressed by the "complete volume curve" of the ventricles. The output, being the product of rate multiplied by amplitude of beat, is greater at rapid rates than it is at slow merely because, owing to the parabolic form of the relaxation curve of the ventricles, the amplitude is decreased pro-

¹ For discussion and references see our previous papers: this Journal, 1913, xxxi, pp. 288 and 352. See also a review by C. G. Douglas in the forthcoming volume of the *Ergebnisse der Physiologie*.

portionately less than the rate is increased. According to our view *the utmost assistance that respiration can afford to the circulation is to maintain a venous pressure sufficient to distend the right ventricle as rapidly as it relaxes and as fully as the duration of diastole allows.*

In the literature this topic has usually been dealt with in connection with those variations of arterial pressure which often accompany the different phases of respiration.² We shall therefore briefly review the principal factors which are supposed to play a part in conditioning these variations, and shall then attempt to estimate the importance of each in the light of our experiments. It is to be noted that we are not here concerned with the vaso-motor element (if any) in the rise and fall of arterial pressure associated with breathing, but solely with the rhythmic changes in the activity of the heart. The factors to be considered include not only the changes in intra-thoracic and intra-abdominal pressure as they may affect the volume of the systolic discharges of the heart, but also the influence of the rate of beat. The ultimate causes of the respiratory variations in the heart rate are not, however, a part of our topic. It is sufficient for present purposes to recognize that, as these rhythmic changes of rate of beat are abolished by cutting the vagi, they are of central origin. Their interest in this paper lies wholly in the fact that they are themselves the causes — and in our opinion normally the principal causes — of variations in the output of the heart.

The method of analysis to be here employed involves the assumption that all conditions affect the right and left ventricles equally. There are theoretical objections to this assumption, but without it discussion of this topic degenerates into mere speculation. In the last analysis variations in the output of the left ventricle depend upon the pressure in the pulmonary veins and upon the character of the receptive relaxation of the left ventricle under different pressures in the pulmonary veins. In respect to both of these factors there is at present an almost entire lack of definite information.

The factors generally supposed to be involved in the respiratory variations of arterial pressure are the heart rate, the negative pressure of the thorax, and the intra-abdominal pressure.

1. Variations in the rate of the heart beat. — In animals under ex-

² TIGERSTEDT: *Ergebnisse der Physiologie*, 1903, ii, 2, p. 528; 1905, iv, p. 481; and 1907, vi, p. 265.

perimental conditions the pulse usually quickens during inspiration and slows during expiration. In man not only this relation but the exact opposite and many intermediate synchronisms likewise occur.³ In the large majority of curves both from man and animals, no matter what the relation of pulse rate to respiratory phase may be, arterial pressure rises with the cardio-acceleration and falls with the period of slower beats. *The relation of pulse to pressure is usually exactly the same as if the vagus were moderately stimulated by artificial means at appropriate intervals.* Surprisingly little attention has been paid to this fact. Some writers have even considered the rise and fall of arterial pressure to be the cause of, instead of being caused by, the variations in the heart rate.

2. *Variations in the negative pressure of the thorax.*—These variations depend upon the relative extent to which the lungs are stretched during inspiration, expiration, and the expiratory pause. Text-books usually contain some such statement as that “The forces which cause the air to flow into and out of the lungs will at the same time and in a similar way cause the blood of the extra-thoracic veins to flow into, through, and out of the intra-thoracic vessels.” The circulation is supposed to be thus affected in three ways: (a) *Variations in the capacity of the pulmonary blood vessels* and in the pressure within them⁴ occur during the successive phases of natural breathing. As Lewis,⁵ however, remarks, “It has never been conclusively demonstrated that such changes have any appreciable influence upon blood pressure.”⁶ It is a fact, which is often forgotten, that the intra-pleural negative pressure can act upon only those small portions of the pulmonary arteries and veins which run outside the lungs. By far the greater part of the pulmonary vascular system, including all of the capillaries

³ PUTZIG: *Zeitschrift für experimentelle Pathologie und Therapie*, 1912, xi, p. 115 (full literature).

⁴ The most recent observations upon these pressures are those of WIGGERS, this *Journal*, 1912, xxx, p. 233; and of WEBER, *Archiv für Physiologie*, 1912, p. 383.

⁵ LEWIS: *Journal of physiology*, 1908, xxxvii, p. 232 (quotation somewhat abbreviated). As nearly as we can gather, Lewis' conclusion (No. 8 on p. 254) regarding the influence of the heart rate is not very different from our own.

⁶ For the opposite view see SAHLI: *Lehrbuch der klinische Untersuchungsmethoden*, 5th edition, Leipzig, 1909, p. 126; and ERLANGER and FESTERLING: *Journal of experimental medicine*, 1912, xv, p. 370.

and the small arteries and veins, is under merely atmospheric pressure to almost the same extent as the blood vessels of the arms and legs. So long as the glottis is open the rise and fall of intra-alveolar pressure, due to the friction met by the air in passing through the trachea, bronchi, etc., during expiration and inspiration are so slight as to be negligible.

(b) *The pericardial pressure* is probably at all times the same, and undergoes the same variations as the (negative) pressure of the intra-pleural spaces. Lewis has found (in agreement with observations previously reported from this laboratory ⁷) that if the heart is enclosed in a plethysmograph and a pressure exerted by forcing a little air into the instrument, the diastolic filling of the ventricles is impeded and arterial pressure falls. He infers that the variations of pericardial pressure play an important part in determining the efficiency of the heart during the successive phases of breathing.⁸ We believe, on the contrary, that the experiments do not adequately imitate natural conditions. During normal life the force which distends the right ventricle in diastole is the difference between the pressure within the thoracic veins and that in the pericardial and intra-pleural spaces. The (negative) pressure of these spaces acts at every instant both upon the veins and upon the heart to an equal degree. In the experiments here cited, on the contrary, the pressure upon the exterior of the heart was much greater than upon the veins. The conditions were somewhat like those of Valsalva's experiment.

(c) *The suction induced in the intra-thoracic veins* by the negative pressure of the thorax has by some writers been supposed to draw the blood onward from the extra-thoracic vessels. If so, the inspiratory increase of the negative pressure would augment the venous supply to the right heart. It seems now to be generally recognized, however, that a suction cannot be transmitted to any considerable distance through veins. They are too readily collapsible; the pressure within them is too low, and the flow is too slow. In purely thoracic breathing in a recumbent subject the jugular veins just above the clavicle become less distended during moderate inspiration and may

⁷ HENDERSON, Y.: this Journal, 1906, xvi, p. 367.

⁸ GROEDEL assigns a somewhat similar influence to a supposed compression of the heart by the pericardial sack due to the pull of the diaphragm during hyperpnœa: *Zeitschrift für klinische Medizin*, 1910, lxx, p. 47.

even collapse during deep inspiration. This indicates that the "top of the venous column" falls. It probably falls nearly as much as the intra-pleural pressure. If so, the "effective venous pressure," *i. e.*, the difference between the pressure in the veins and that in the intra-pleural spaces, is probably not much greater during inspiration than during expiration. Furthermore, as we have shown in a previous paper (*loc. cit.*), normal conditions maintain an effective venous pressure during all phases of quiet breathing sufficient to insure the filling of the right ventricle as rapidly as it relaxes. The efficiency of the heart is therefore maximal for each particular rate of beat even during expiration, and mere increase of venous pressure during inspiration, apart from acceleration of the rate of beat, cannot augment the output.

3. **Intra-abdominal pressure.**⁹ — This force has undoubtedly under some conditions an important influence upon venous pressure, and the supply of blood to the right heart, although we regard it as being normally merely an auxiliary to the changes of heart rate. In quiet breathing of the abdominal type the inspiratory contraction of the diaphragm tends to compress the abdominal vessels. If at the same moment the heart beat is quickened, the increased venous supply from the abdomen insures the effective venous pressure against falling below the critical value. In an athlete panting after a contest powerful and rapid expiratory contractions of the abdominal muscles occur, and venous pressure is thus raised considerably. According to the conception of the heart's behavior held by Zuntz, Plesch, and Krogh, the diastolic filling and systolic discharge of the ventricles may thus be increased to a maximum in spite of the rapid rate of beat. We have shown, on the contrary, that owing to the shortness of diastole the amplitude of the heart strokes at such times must be smaller than during bodily rest. According to our view the value of the high venous pressure during physical exercise lies in the fact that the tonus of the heart is greatly increased by tachycardia and the ventricles are thus rendered less readily distensible than at slow rates and low tonus. The high venous pressure serves to distend the right ventricle with normal rapidity and, so far as the duration of diastole allows, to a normal extent in spite of the high tonus. This view is in agree-

⁹ For full discussion and literature see EMERSON, H: Archives of internal medicine, 1911, vii, p. 754.

ment with the recent observations of Hooker¹⁰ upon venous pressure in man during exercise.

Methods of observation and results. — In the course of investigations reported in previous papers upon the influence of venous pressure and of the cardiac nerves upon the efficiency of the heart we found that we had almost ideal conditions for analyzing the respiratory influences also. The subjects (dogs) were morphinized and etherized. The thorax was opened by cutting all of the costal cartilages. The negative pressure due to the elasticity of the lungs was thus absolutely eliminated. The subjects were allowed to maintain spontaneous respiration of slightly compressed air. The arrangement was essentially that of Brauer's Ueberdruckverfahren.¹¹ The cannula tied into the trachea was connected with a T-tube leading on the one side to a weighted gasometer into which air was continually pumped, and on the other to a Müller valve set to a sufficient depth to keep the lungs distended — usually about 8 cm. of water. Thus the subject inspired from the tank and expired through the valve. There was also a dead space (a large tube) of adjustable volume between the cannula and T-tube, so that more or less re-breathing could be maintained. Arterial pressure was recorded from the carotid by a Hürthle manometer (old style); and the behavior of the heart was inscribed below it by a large tambour connected with a plethysmograph enclosing only the ventricles. Venous pressure was measured by means of a special form of manometer in the right jugular. The instant at which inspiration began, and that at which it ended and expiration set in, were noted on the record by hand.

In these experiments the observation which particularly merits emphasis is that *the variations in arterial pressure are of the same character and bear the same time relations to the phases of respiration in an animal which is breathing spontaneously after the chest has been opened as in a subject with the thorax intact.* So far as we are aware this fact has not previously been emphasized. It goes far to show that the changes in pericardial pressure and in the suction of blood into the intra-thoracic veins (as discussed above in section 2 under *b* and *c*) are much less important than has generally been supposed in rela-

¹⁰ HOOKER: this Journal, 1911, xxviii, p. 235.

¹¹ BRAUER: Mitteilungen aus den Grenzgebieten der Medizin und Chirurgie, 1904. xiii, Art. xviii.

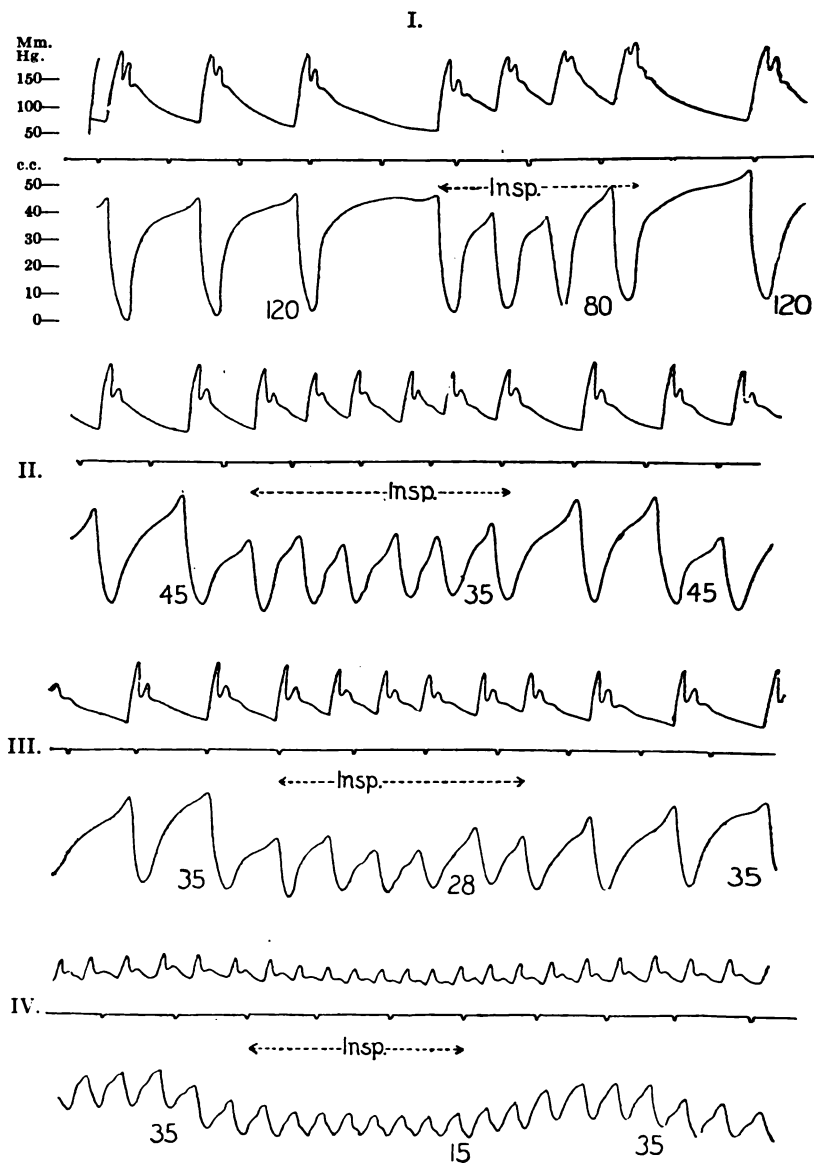
tion to the respiratory variations in the velocity of the blood stream. If these factors were necessarily involved in the rise and fall of arterial pressure during normal breathing, variations of pressure could not occur under the conditions of our experiments. As the thorax was open, there were no variations of pericardial pressure or venous suction, and yet respiratory variations of arterial pressure of practically all known types were obtained.

Comparison of the arterial pressure record with the volume curve of the heart tends to eliminate also the (supposed) variations in the capacity of the pulmonary vessels (see section 2 *a* above) as a factor in the rhythmic changes of the heart's activity. Unlike the conditions under artificial respiration, the intra-alveolar pressure in our experiments was nearly the same in inspiration and in expiration. Study of the graphic records shows that *the variations in the output per second are explicable mainly on the basis of variations in the rate of heart beat supplemented under some conditions by changes of intra-abdominal pressure.*

Examples of the data upon which these statements are based are reproduced in Figs. I to IV. All of these records were obtained from the same animal. The differences between them were due to a progressive lowering of venous pressure induced by repeatedly withdrawing small quantities of blood. The evidence which these curves afford is summarized in Table I.

Fig. I shows practically normal conditions. As the rate of beat becomes quicker, more blood is pumped into the aorta and arterial pressure rises correspondingly. As the rate becomes slower, the output per second is lessened and the pressure falls again. The volume and pressure in the venous stream to the right heart are sufficient to distend and fill the ventricles as rapidly as they relax in diastole. Each systolic discharge is the largest that the duration of the preceding diastole allows the heart to make. During the period of acceleration the abbreviation of the diastoles lessens the volumes discharged by the individual systoles, but the output per second (*i. e.*, the rate multiplied by the amplitude of stroke) is increased. During the periods of slower heart rate the longer diastoles allow a marked increase in the size of the strokes, but the flow per second is decreased. Arterial pressure rises and falls accordingly.

So far as we can ascertain, these relations are essentially those which



FIGURES I to IV. — About one half original size. From a dog of 9.5 kilos. Arterial pressure, time in seconds, and ventricular volume curve. A natural respiration of air at a pressure of 80 mm. of water was maintained. The dotted arrows indicate the beginning and end of inspiration. The figures below the volume curves give the venous pressure in millimetres of saline. After each record the animal was subjected to a hæmorrhage sufficient to produce a distinct lowering of venous pressure. In all cases mean arterial pressure is seen to rise and fall in close correspondence with increase and decrease in the output of the left ventricle per second. The data obtainable from the curves are summarized in the table.

In I venous pressure, owing to hypercapnia and a previous intravenous infusion of saline, was continually above the critical value needed to fill the ventricles as rapidly as they relaxed in diastole. All of the volume curves in this record are therefore superimposable. The variations in the output per second during inspiration and expiration were determined wholly by the rate of beat and its influence upon the complete volume curve.

In II venous pressure was somewhat below the critical value during expiration and even lower during inspiration. Accordingly the amplitude of beat during inspiration is so reduced that in spite of the accelerated rate the output is only slightly greater than during the slower pulses of expiration. While mean and diastolic arterial pressures rise during inspiration, the systolic pressure (*i. e.*, the tops of the pulse waves) falls.

In III the venous pressures are still lower, and the inspiratory beats fall so far short of superimposability in relation to the slower expiratory strokes, that the output per second is practically the same in all phases of breathing. Mean arterial pressure is practically uniform, although the pulse pressure varies considerably.

In IV arterial pressure falls during the rapid but extremely small beats of inspiration, and rises in expiration. The increase of venous pressure which caused this rise was due to forcible expiratory contractions of the abdominal muscles. Their effect on the amplitude of beat and thus on output and arterial pressure is distinctly shown. In the first two figures on the contrary, the rise of venous pressure during expiration was mainly due to the decrease in the pumping activity of the heart by the slowing of the rate of beat.

occur also under all normal conditions. When the cardio-acceleration accompanies or closely follows inspiration, as is usually the case in animals and in our experience generally also in man, the "inspiratory rise of arterial pressure" results. When the cardio-acceleration comes

TABLE I.

TABLE OF DATA OBTAINED FROM THE CURVES OF FIGS. I TO IV SHOWING THE RELATION AND CONSEQUENT VARIATIONS IN ARTERIAL PRESSURES (SYSTOLIC, DIAS-

Number of record.	Respiratory phase.	Venous pressure.	Heart rate beats per second.	Systolic discharge. ¹
		mm. of saline.		c.c.
I	{ Insp.	80	1.2	19
	{ Exp.	120	0.6	24
II	{ Insp.	35	1.5	13
	{ Exp.	45	0.9	20
III	{ Insp.	28	1.4	10
	{ Exp.	35	0.8	18
IV	{ Insp.	15	2.8	4
	{ Exp.	35	2.0	8

¹ The systolic discharge of the left ventricle is taken as one half the

during expiration, as is not infrequent in man, an "expiratory rise" occurs. *It is not the phase of respiration, but the heart rate, which determines the rise and fall of arterial pressure, so long as venous pressure is above the critical value.*

When venous pressure falls below the critical value, as in Figs. II, III, and IV, the conditions become more complex. The size of the strokes now depends not only on the duration of the diastoles preceding them, but also on variations of venous pressure. When the venous stream to the right heart is not very much below the full requirements and no very decided contractions of the abdominal muscles occur, the result is to decrease (Fig. II), or even to prevent (Fig. III) the variations of mean arterial pressure. Thus in ~~the~~ ~~the~~ systolic pressure falls during inspiration about as in

pressure rises. The pulse pressure is much greater during expiration than during inspiration because the individual strokes of the heart are larger, but the output and mean pressure are practically uniform.

When, however, the circulation has been so far reduced by hæmor-

TABLE I.

OF VENOUS PRESSURE, RATE OF BEAT, OUTPUT OF THE LEFT VENTRICLE PER SECOND, TOLIC, MEAN, AND PULSE PRESSURES) DURING SPONTANEOUS BREATHING.

Output per second.	Rise or fall of arterial pressures.			
	Systolic.	Diastolic.	Mean.	Pulse.
22.8	+	+	+	-
14.4	-	-	-	+
19.5	-	+	+	-
18.0	+	-	-	+
14.0	-	+	Uniform	-
14.4	+	-	"	+
11.2	-	-	-	-
16.0	+	+	+	+

amplitude of the volume curve measured by means of the scale of c.c.

rhage or shock that the medullary centres are affected, the respiratory centre responds by marked expiratory contractions of the abdomen. These contractions temporarily increase venous pressure to such an extent that, although cardio-acceleration occurs during inspiration, the output is greater and the arterial pressure higher during expiration (Fig. IV.). Under these conditions arterial pressure would rise with expiration and fall during inspiration even if the heart rate were uniform. Such an inversion of the common relation of respiration to arterial pressure is frequently seen in animals in shock. The form of breathing here shown to cause it occurs also in human subjects after extensive hæmorrhage or in circulatory shock. It is characterized by quick shallow inspirations and prolonged sighing or groaning expirations, the so-called "expiratory grunt." Its value lies in its effect on venous pressure.

Confirmatory observations.—The following observations made upon subjects in which the thorax was intact tend to confirm our opinion that, apart from variations of the heart rate synchronously with respiration, and apart from changes of intra-abdominal pressure when the venous supply is below normal, the movements of breathing have in reality no considerable influence in accelerating the blood stream:

1. When animals (dogs and cats) are asphyxiated, it frequently happens that several seconds after the heart has stopped beating one or more deep gasping inspirations are drawn. If these movements caused any onflow of blood into the arterial system (as they should according to the generally accepted theory), the effect should be perceptible as at least a slight rise in the record afforded by a delicate spring manometer connected with the carotid. In fact, however, unless the heart also executes a beat, the manometer at such times describes a straight line.

2. If the movements of natural breathing really tend to pump blood onward, forcible artificial respiration should also have this effect. A bellows with the inlet valve plugged was connected airtight to the trachea of a dog soon after it had been killed with chloroform. The bellows was worked vigorously so as to produce alternately as great positive and negative pressures as possible in the lungs. A spring manometer connected with the carotid showed not the slightest discharge of blood into the aorta. The only result of the experiment was an extraordinary emphysema in the dead body.

3. In making arterial pressure measurements upon normal men by means of a sphygmomanometer the reading is usually nearly the same, no matter whether taken during inspiration or expiration. In normal subjects it is only when the pulse rate varies with the phases of respiration (as during sleep and especially in children) that any considerable variations in arterial pressure occur. In some of the curves published by previous investigators we find that they have overlooked variations in the pulse rate sufficient to account for the observed rise and fall of arterial pressure.¹² The rhythmic changes of pressure in a normal subject during Cheyne-Stokes breathing under low barometric pressure also synchronize with the alterations in the heart rate.¹³

¹² See for instance ERLANGER and FESTERLING: *Loc. cit.*, fig. 5.

¹³ DOUGLAS, HALDANE, HENDERSON, and SCHNEIDER: Report of Expedition to Pike's Peak, *Philosophical Transactions of the Royal Society*, 1913, p. 83.

CONCLUSIONS.

The primary object of this paper is to determine to what extent the movements of respiration may act as an auxiliary to the heart in accelerating the blood stream. We conclude that the utmost assistance that respiration can afford is the maintenance of a venous pressure sufficient to distend the right ventricle as rapidly as it relaxes and as fully as the duration of diastole allows. These are merely the ordinary normal conditions without which the heart action becomes subnormal. Neither hyperpnœa nor any other influence that we have been able to test is capable of inducing such enormous systolic discharges at rapid rates of heart beat as Zuntz, Plesch, Krogh, and their collaborators believe to occur during vigorous physical exercise.

In regard to the respiratory variations of arterial pressure we hold (contrary to the general opinion) that the influences of the rhythmic changes of the negative pressure of the thorax upon the thoracic veins, upon the heart, and upon the pulmonary blood vessels are in reality of no considerable importance in relation to the velocity and volume of the arterial blood stream.

Under normal conditions of venous supply the respiratory variations of arterial pressure are mainly due to corresponding alterations in the heart rate. A rise of pressure is produced by the period of cardio-acceleration, no matter whether this period occurs in inspiration or in expiration.

When, on the contrary, venous pressure is below normal and the heart is therefore acting with less than full efficiency, as often occurs in animals under experiment, the forcible expiratory contractions of the abdomen may momentarily increase the venous supply to the right ventricle to such an extent that the output of the left ventricle is augmented to a more nearly normal volume. Arterial pressure may thus be raised during expiration even though the rate of heart beat is more rapid during inspiration. This auxiliary action of respiration is important after hæmorrhage and in circulatory shock.

The principal facts here reported are: (1) During spontaneous breathing under "positive pressure" after pneumothorax the varia-

tions of arterial pressure are of precisely the same character as when the thorax is intact. (2) Neither spontaneous nor artificial respiration is capable of causing any appreciable flow of blood from the veins through the pulmonary vessels into the aorta when the heart is not beating.

THE BLOCKING OF NERVE IMPULSES IN THE FROG.

By CHARLES M. GRUBER.

[From the Physiological Laboratory of the University of Kansas.]

BERNSTEIN,¹ in 1877, used the tripolar block² to keep the impulses from passing to the muscles in his experiments on nerve fatigue. Ioteyko³ used it in studying the resistance of medullated nerve centres to fatigue. Schenck,⁴ in watching Achelis⁵ perform his experiments with the tripolar electric stimulating method, noticed that under certain conditions the irritability and nerve conduction were decreased so that no contraction of the muscle resulted upon stimulation of the nerve. These observations he used to substantiate the theory given in one of his previous papers. Ludwig Pflücker,⁶ upon Schenck's suggestion, employed the tripolar method to block the cranial impulses in the vagus. Fröhlich⁷ employed it for blocking impulses in both warm and cold blooded animals, and he also corroborated Pflücker's work in excluding the vagus impulses. Since the publication of his paper, as far as I have been able to ascertain, no further investigations have been made with this block.

The object of my experiments was to investigate the practical

¹ BERNSTEIN: *Archiv für die gesammte Physiologie*, 1877, xv, p. 289.

² For the tripolar method used for stimulation, not for blocking, see FILEHNE: *Deutsches Archiv für Medizin*, 1870, vii; DANILEWSKY: *Centralblatt für Physiologie*, 1895, ix, p. 390; SCHATERNIKOW: *Centralblatt für die medicinischen Wissenschaften*, 1895, xxvi, p. 449; WERIGO: *Archiv für die gesammte Physiologie*, 1899, lxxvi, p. 517; ACHELIS: *Archiv für die gesammte Physiologie*, 1905, cvi, pp. 329-368.

³ IOTAYKO: *C. Richet's Dictionnaire de physiologie*, 1904, vi, p. 151.

⁴ SCHENCK: *Archiv für die gesammte Physiologie*, 1905, cvi, p. 368.

⁵ ACHELIS: *Loc. cit.*

⁶ PFLÜCKER: *Archiv für die gesammte Physiologie*, 1905, cvi, pp. 372-401.

⁷ FRÖHLICH: *Archiv für die gesammte Physiologie*, 1906, cxiii, pp. 418 and 433.

value of the tripolar⁸ block against impulses caused by different kinds of stimuli, and to compare its efficiency with the blocks in other methods.

The experiments were performed upon frogs of the species *Rana pipiens*.

METHOD.

The frogs were fastened upon Hall's combination frog-board, fitted with an insulating glass plate. For the stimulating current I used a Harvard inductorium graduated in one sixteenth inches. The primary coil was connected through a duBois-Reymond key and one Leclanché cell of 1.2 voltage. Unless otherwise stated, the stimulus was a faradic current. Very small insulated platinum electrodes were used in the secondary circuit when the nerve was stimulated directly, and cable platinum electrodes when stimulating the foot for strychnine reflexes. In a few of the experiments a resistance was introduced in the secondary current as a control. Resistances varying from 4000 to 225,000 ohms were interpolated through a resistance box or a ground-glass plate with lead-pencil lines. In most of my later experiments I omitted the resistances because they proved unnecessary.

Fröhlich's⁸ method of connecting the non-polarizing electrodes to the nerve was used, except that the zephyr fibres were moistened in normal salt solution instead of Ringer's solution. The three non-polarizable electrodes were placed in the circuit so that the middle one was joined to the two negative poles, and each outer electrode to one of the positive poles of the batteries. Consequently the middle electrode always carried twice the voltage of either of the outer, positive electrodes.

One of the five following groupings of voltages was used in these experiments, namely:

2 (1.4)	2 (2.8)	2 (4)	2 (10.3)	2 (12.8) volts.
2 cells	4 cells	6 cells	12 cells	18 cells.

As the strength of the cells may vary from day to day, their voltage was tested with a volt-metre before each experiment. This was done to make sure that both positive boots were of equal strength.

⁸ FRÖHLICH: *Loc. cit.*

It may be questioned whether part or all of the impulses are blocked after electrical stimulation, even when no contraction results, because, possibly, they may not be strong enough to cause a contraction. To ascertain if that were true galvanometric readings were taken. It was found that when a block was obtained no deflection of the galvanometer occurred. When there was no block, a deflection was sure to result. This shows that no impulses passed the block.

BLOCKING AGAINST THE ELECTRICAL STIMULUS.

The tripolar block. — The object of this series of experiments was to determine the strength of current required to block a weak electrical stimulus which causes a contraction.

The frog was pithed, or put under ether anæsthesia. The sciatic nerve was exposed as far as the spinal cord and insulated from the surrounding tissue, either by means of a double rubber dam made of sheet rubber or by a glass plate. The split zephyr fibres were folded around the nerve as close to the muscle as possible in order to place the stimulating electrodes outside of the anelectrotonic area. Great care was taken to place the outer fibres equally distant from the middle one — 0.5 cm. is the best distance — and not to have any moisture between them. In a few cases the last electrode — the one next to the muscle — was placed on the gastrocnemius muscle instead of on the nerve. This also proved to produce an efficient block. The bipolar method was also tried, but the tripolar was more efficient.

The insulated stimulating electrodes were then placed on the nerve as far as possible from the block electrodes without stretching or pressing the nerve. In every case the stimulating current was the weakest possible which would cause tetanus and a maximal contraction of the muscle. This was found to be, in the majority of cases, without a resistance in the secondary coil, when the latter was 12.5 cm. from the primary coil.

From a large series of experiments it was ascertained that a strong tetanic contraction of the muscle resulting from a faradic stimulation instantly ceased the moment that the block circuit was closed. The instant that the block circuit was broken a contraction resulted upon again stimulating the sciatic nerve. While the nerve muscle preparation was fresh a large voltage was required in the block circuit; usually

2 (10.3) volts, in a few cases 2 (4.2) volts were sufficient, but in some as much as 2 (12.8) volts were necessary (see Fig. 1). The block was often efficient against maximal electrical stimuli. As controls to prove the efficiency of the block, liquid air or a freezing mixture of NaCl and ice was used for freezing, and in a few of the experiments magnesium sulphate or cocaine was used to narcotize the nerve.

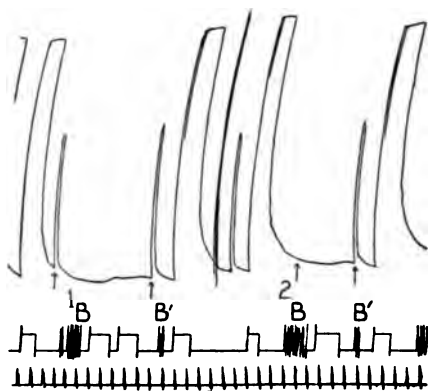


FIGURE 1. — The uppermost curve shows the contraction of the gastrocnemius muscle of the frog, the middle curve records duration of stimulation and duration of block, the lowest curve is the time in seconds. Block current tested 2 (10.6) volts. Nerve intact. Faradic stimulation of left sciatic nerve. 1. 4000 ohms resistance in stimulating current. 2. No resistance. Stimulating current 7.5 cm. from the block electrodes. Vertical arrows indicate the duration of the block.

Liquid air. — When a fibre of absorbent cotton dipped in liquid air was applied to the intact nerve, the muscle contractions due to electrical stimuli were shut out in every case, and they remained so as long as the nerve was frozen. As soon as the nerve was no longer frozen, the contractions again appeared. As long as the nerve remained intact, this procedure could be repeated an indefinite number of times without much injury to the conductivity of the nerve impulses. But when the nerve was cut and then frozen, or cut after it had been frozen, the muscles would not again respond to electrical stimuli, even after a

half-hour's rest, until the stimulating electrodes were moved beyond the previously frozen part of the nerve.

Sodium chloride and ice. — To ascertain whether liquid air was peculiar in causing the death of the nerve when cut, a control with a freezing mixture was made. Sodium chloride and ice were placed in a glass tube. This tube was then carefully placed on the nerve. With this block the results were the same as those obtained with the liquid air block.

Cocaine. — The efficiency of the tripolar electrical block was also compared with that of the cocaine block. The time required to produce the cocaine block was very long, often from twenty-five to forty-

five minutes. In no case, in my experiments, did the nerve return quite to normal, even after waiting from one half to three quarters of an hour. The stimulating current in every case was strengthened by moving the secondary coil 1 mm. to 3 mm. nearer the primary coil, showing a decrease in irritability or conductivity in nerve tissue, even forty-five minutes after the block was removed. This is not so with the electrical block. The nerve, whether intact or cut, retains its normal functions, whether blocked once or many times, unless the stimulating current is so strong that it paralyzes the nerve⁹ completely.

Magnesium sulphate.—The results here were the same as with the cocaine block.

In the above series of experiments the efferent impulses caused by direct electrical stimulation of the nerve were blocked without apparent injury by the tripolar method, and with but slight injury by either liquid air or a freezing mixture, and with more or less injury by cocaine or magnesium sulphate. Sectioning the nerve after freezing causes a destruction that is avoided by the other methods.

In the next series of experiments I shall show that reflex efferent impulses caused by stimulating the opposite foot can also be blocked. For these experiments strychnine frogs were employed.

BLOCKING IN THE STRYCHNINE FROG.

Strychnine tetanus.—The frog was weighed and then prepared for the experiment as above. For each 15 gm. of body weight, 1 minim of 0.1 per cent strychnine sulphate solution was injected into the dorsal lymph sac. The strychnine tetanus which soon resulted was blocked with a strong electrical current, 2 (10.6) volts. In a few cases 2 (4.2) volts blocked the tetanus impulses during the whole interval of time that the block current was introduced.

Efferent impulses.—Without disturbing the block electrode, by stimulating the opposite foot either electrically or mechanically, a reflex response resulted which was blocked when the block circuit was closed. Usually a strong block of 2 (10.6) volts was required, but occasionally 2 (4.2) volts sufficed (see Fig. 2).

⁹ HYDE: this Journal, 1906, xvi, p. 368.

In a few cases the fore legs were stimulated instead of the opposite hind leg. The block was so efficient that the fore legs could be severed without producing the usual reflex. In every case 2 (10.3) volts was required to block these impulses. When the block circuit was opened,

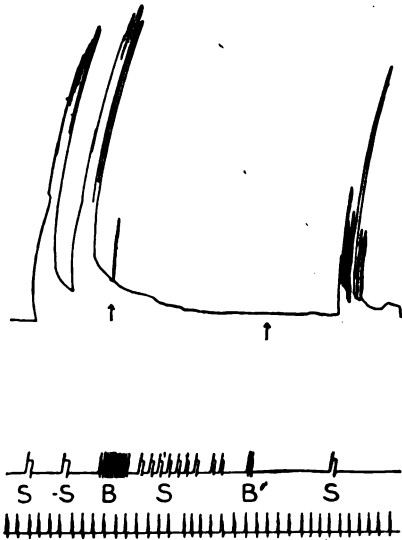


FIGURE 2. — Efferent impulses blocked. Block current of 2 (12.8) volts was placed on left sciatic nerve. Stimulated the skin of opposite foot with a faradic current. Vertical arrows indicate the duration of block.

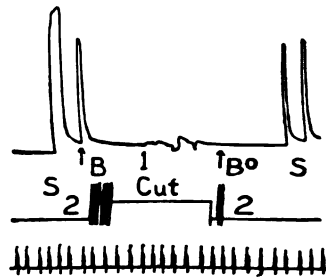


FIGURE 3. — The left front foot was severed at the last joint. Block current strength, 2 (10.2) volts. Mechanically stimulated the toes of front foot by pricking them with a pair of scissors. Vertical arrows indicate the duration of block.

a prick or touch on the fore legs before they were cut would cause intense contractions (Fig. 3).

Afferent impulses. — The zephyr fibres were then placed on the leg opposite to the one attached to the recording lever. When the leg was stimulated, either by touching its skin with a pencil point or by a minimal electrical stimulation, a reflex contraction resulted in the other leg, but if the block circuit was closed and the foot stimulated again with the same strength stimulus no contraction resulted. No muscle of the body except those in the stimulated foot contracted. In almost every case 2 (1.4) or 2 (1.3) volts proved efficient. These results show that the afferent impulses are blocked with a much weaker current than are the efferent (see Fig. 4).

When the sciatic nerve was stimulated directly, either by a weak interrupted or a make and break current, instead of stimulating the skin of the foot, the result was the same as above described, and the same kind of curves were obtained as shown in Figs. 2 and 4.

Liquid air, sodium chloride, and ice.—As a control for the efficiency of the electrical block, I repeated the above experiments on a strychnine frog, but used liquid air, and a freezing mixture instead of the electrical block. I applied them in the way described above, and blocked both the strychnine tetanus and the afferent and efferent impulses. The time required for the complete functions of nerve conduction to return after freezing was from half an hour to an hour.

SUMMARY.

1. Nerve impulses produced by electrical or other kinds of stimuli can be blocked by the tripolar electric block.
2. The minimal strength of current required to block the efferent impulses varies with the condition of the frog. 2 (10.2) volts, as a rule, proved efficient.
3. In the intact as well as in the cut nerves, the block acts similarly.
4. Strychnine tetanus can be blocked.
5. The fore leg can be severed without causing a contraction or electrical variation in the leg to which the block is applied.
6. Afferent and efferent impulses, set up by an electrical or a mechanical stimulation of the foot of a strychnine frog, can be blocked.
7. The afferent impulses are cut out by a small voltage—2 (1.4) to 2 (2.8) volts—whereas the efferent require from 2 (10.3) to 2 (12.8) volts.

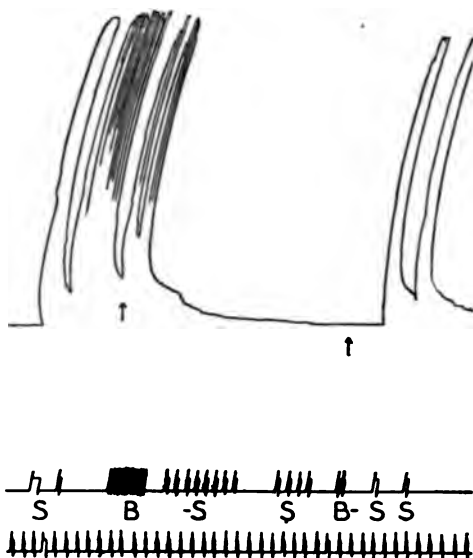


FIGURE 4.—Afferent impulses blocked. The block current of 2 (1.4) volts was placed on the right sciatic nerve. Faradic stimulation of the same foot. Left gastrocnemius muscle attached to the writing lever. Vertical arrows indicate the duration of block.

8. The electrical tripolar block is superior to the other blocks investigated in that it excludes all impulses and may be employed a long time without impairing the conductivity of the nerve. It also affords a ready means for excluding the afferent impulses in a mixed nerve trunk.

I wish to thank Dr. Ida H. Hyde for suggesting the subject of this investigation and for her kind assistance during my experiments.

THE ACTION OF VARIOUS INFLUENCES UPON THE RHYTHMICITY OF THE NODAL, SINUS, AND AURICULAR MUSCULATURE OF THE MAMMALIAN HEART.

By V. H. K. MOORHOUSE.

[From the Pharmacological Laboratory of Washington University Medical School, St. Louis.]

THE determination of the region which regulates the beat of the heart has been attempted in different ways. One method has sought the point at which influences affecting rhythm exert a maximal effect upon the rate of the heart. The rhythmicity may be influenced by temperature changes and by drugs. The purpose of the present work is to study the effect of these agents upon the various kinds of musculature found in the auricle.

The right auricle, and particularly that part of it which is traceable developmentally to the sinus venosus, has been indicated as the region which takes the most active part in the reactions to these agents. Here the musculature shows the highest power of initiating and of responding to impulses, and temperature changes produce here their maximal effect on the whole heart. These facts point to a strong probability that the various substances which produce changes in heart activity find in this region a specially receptive and reactive area. Since the muscle tissue in this locality is the most highly rhythmical, it becomes probable that drugs affecting muscular tissue would exert here a maximum influence. In the work of Loeb¹ we have experimental proof that such is the case. Loeb shows that drugs applied to the sinus produce their effect on the heart more rapidly and in less concentrated solution than when applied to any other portion. The specific muscular tissue of the sino-auricular node has been indicated as the point at which greatest variation of heart rate can be elicited by changes of temperature.

¹ LOEB, OSWALD: *Verhandlung des Kongresses für innere Medizin*, 1911, xxviii, p. 334.

Whether the nodal musculature is especially reactive to pharmacological influences has not as yet been studied. The abundance of nervous elements in the tissue of the sinus would point to the probability that drugs having affinity for nerve endings or cells should also produce their effect on the heart mainly by an action on this region. In this regard the work of Flack² has established that drugs which depress or stimulate vagus endings or ganglion cells are most effective here. His observations will be dealt with in more detail in the subsequent sections. The investigations of Marchand and Meyer,³ with the object of ascertaining the principal areas of intracardial innervation by the vagus also demonstrate a selective action of nicotine on the sinus.

It is naturally thought that the area of musculature which regulates the beat of the heart should have a distinctive power of rhythmicity and of reactivity to such influences as affect rhythm. From a consideration of the experimental work dealing with the effect of such influences on the heart, it is apparent that the main part in such phenomena as appear may be ascribed, not to any particular portion of the sinus region, but to the whole highly rhythmical area. If a method could be used by which the comparative reactions of various parts of the heart and especially of the sinus region could be studied, it seemed desirable to carry out such an investigation. The method described by Erlanger⁴ of using isolated strips from the cat's heart suggested itself as being a favorable way of comparing the reactions of rhythmical areas. Since the excised strips from the cat's auricle are spontaneously rhythmical when suspended in Ringer solution, it becomes possible to observe the reactions of various portions of the sinus region. In experiments done on the intact heart results obtained from local measures are attributable to an indefinite area. Thus, when temperature changes occur in the region between the great veins of the mammalian heart, it seems improbable that a response can be ascribed to a more definite area than that occupied by various types of tissue. Changes of temperature may spread to surrounding tissues, and there produce an effect which may enhance or partially annul a

² FLACK, MARTIN: *Journal of physiology*, 1910, xli, p. 64, and *Archives internationales de physiologie*, 1911, xi, p. 127.

³ MARCHAND and MEYER: *Archiv für die gesammte Physiologie*, 1912, cxlv, p. 401.

⁴ ERLANGER: *this Journal*, 1910, xxvii, p. 87.

local effect. Solutions are readily spread on the surface of the auricle and it is not probable that resulting phenomena could be traced to a definite area of the sinus. In experiments in which local applications are made to the intact heart it is probable that a local influence not necessarily affecting areas of impulse production may change the activity of the entire auricle. The reactions of isolated strips are independent of such confusing changes from other areas. Their behavior gives us an indication of the part taken by the different types of musculature in the regulation of the heart beat.

In these experiments strips were excised from the perfused cat's heart and suspended in a bath of Ringer solution, oxygenated and kept at a constant temperature. The contractions of the strips were recorded by means of silk thread and light heart levers. The arrangement of the apparatus was as described by Erlanger⁴ and referred to by the author⁵ in a previous paper. The Ringer bath had a capacity of 600 c.c. and was provided with thermometer, oxygen cannula, out-flow siphon, and a stirring rod. The solution in the bath could thus be kept of uniform character. Drugs were added to the bath in small amounts of their solution in Ringer, thus assuring little or no change in temperature or volume of the fluid. The strips could be immersed in fresh Ringer solution whenever desired. The Ringer bath was kept constantly stirred, especially after injection of drugs.

The strips employed in the experiments were excised from the regions of the heart shown in the diagram (Fig. 1). The strip *A* was removed from the node-bearing area of the right auricle. It was excised carefully so as to include the whole of the sino-auricular node, and therefore consisted of large portions of caval, sinus, and auricular musculature. If the specialized tissue of the node were to possess a

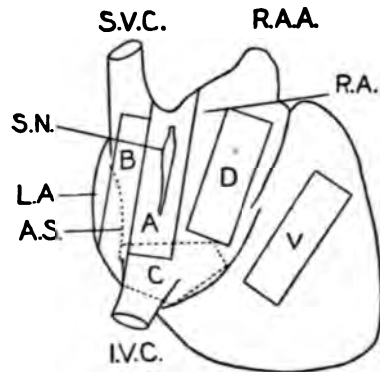


FIGURE 1. — Diagram of cat's heart. *A, B, C, D, V*, strips used in the experiments. *S.V.C.*, superior vena cava. *R.A.*, right auricle. *R.A.A.*, right auricular appendage. *S.N.*, sino-auricular node. *L.A.*, left auricle. *A.S.*, auricular septum. *I.V.C.*, inferior vena cava.

⁵ MOORHOUSE: this Journal, 1912, **xxx**, p. 358.

peculiar susceptibility to changes in the Ringer bath, the strip *A* should exhibit such properties to a greater extent than other strips. The strip *B* was removed from the position shown in the diagram, with the object of obtaining a portion of sinus tissue without any specific nodal tissue. This strip was necessarily composed mainly of tissue from the inter-auricular septum and left auricle with masses of fibrous and fatty tissue. The strip *C* was excised from the coronary sinus region and contained tissues from the septum, small portions of the right and left auricles, and much fibrous and fatty tissue from the auriculo-ventricular boundary. Other strips used were *D*, from the wall of the right auricle, the excised right auricular appendix, and *V*, a strip from the wall of the right ventricle.

Two strips were compared at a time in an experiment. In most of the experiments *A* and *B* were used, next in frequency *A* and *C*. These three strips, representing, as we know, the most rhythmical areas of the auricle, beat spontaneously when immersed in the Ringer bath. They gave a vigorous contraction and continued beating regularly for long periods. Towards the end of experiments, and under the influence of certain drugs, they developed blocks of varying character. The comparison of the grade of spontaneous rhythmicity in this series of experiments is interesting (Table I). This table is based on the results of 65 observations in which the reactions of the strips were compared. It is to be noted that the strips *A*, *B*, and *C* possess very similar grades of rhythmicity, judging by the rate assumed in the Ringer bath, at temperatures of about 35° C. and before any drug had been added to the bath. The results obtained with the use of various measures are given in the subsequent sections. The effects of various changes in the Ringer bath have been taken as typical when produced early in the course of an experiment. That the phenomena agree in general with the reactions of the perfused isolated mammalian heart will appear from these results. The differences in reaction of the various strips have been carefully considered, and such tracings as are published show effects which have been produced in numerous experiments.

I. CHANGES OF TEMPERATURE.

The older investigations have established that the heart is readily influenced by changes of temperature occurring in the sinus region.

It is necessary merely to refer to the earlier experiments, of which those of McWilliam ⁶ and Adam ⁷ are the most important. Since the discovery of the sino-auricular node investigators have transferred to the specific musculature the properties before ascribed to the sinus. Brandenburg and Hoffmann ⁸ find that the rate of perfused

TABLE I.

SHOWING COMPARATIVE RHYTHMICITY OF THE VARIOUS STRIPS¹ USED IN THE EXPERIMENTS.

	A.	B.	C.	D.	R. A. A.	V.
Average normal rate in 10".	20.2	18.9	21.0	8	11	6
Percentage of experiments in which spontaneous beat did not appear.	7.5	12.5	7.5	50.0	Beat only in 1 exp.	Beat only in 1 exp.
¹ Strips of cardiac muscle as shown in Fig. 1.						

mammalian hearts can be influenced only in one particular place by application of cold. This place corresponds with the position of the sinus node. When the nodal region is clamped off, the impulse arises from some other area, but application of cold now does not influence the rate. Ganter and Zahn ⁹ ascribe effect of temperature changes to nodal tissue. Their experiments were done on the mammalian heart *in situ*, and their observations are therefore necessarily limited to the external region of the auricle. By this means they compared the acceleration produced by application of warm cannulas to various points along the sulcus terminalis. The only points which they compared with places overlying the nodal tissue were those on the wall of the right auricle and on the veins. They state that the greatest variations of rate for heating or cooling occur when such changes affect the region corresponding with the thickest part of the node. In a more recent paper Zahn ¹⁰ described an investigation of the deeper areas by the

⁶ McWILLIAM: Journal of physiology, 1888, ix, p. 167.

⁷ ADAM: Archiv für die gesammte Physiologie, 1906, cxi, p. 607.

⁸ BRANDENBURG and HOFFMANN: Medizinische Klinik, 1912, p. 16.

⁹ GANTER and ZAHN: Archiv für die gesammte Physiologie, 1912, cxlv, p. 335.

¹⁰ ZAHN: Zentralblatt für Physiologie, 1912, xxvi, p. 495.

temperature method. He finds that warming or cooling of the coronary sinus region causes marked variations in the rate of the heart, after the sinus node has been destroyed. In such experiments he finds the A-V sequence unchanged when the sinus node is eliminated. This

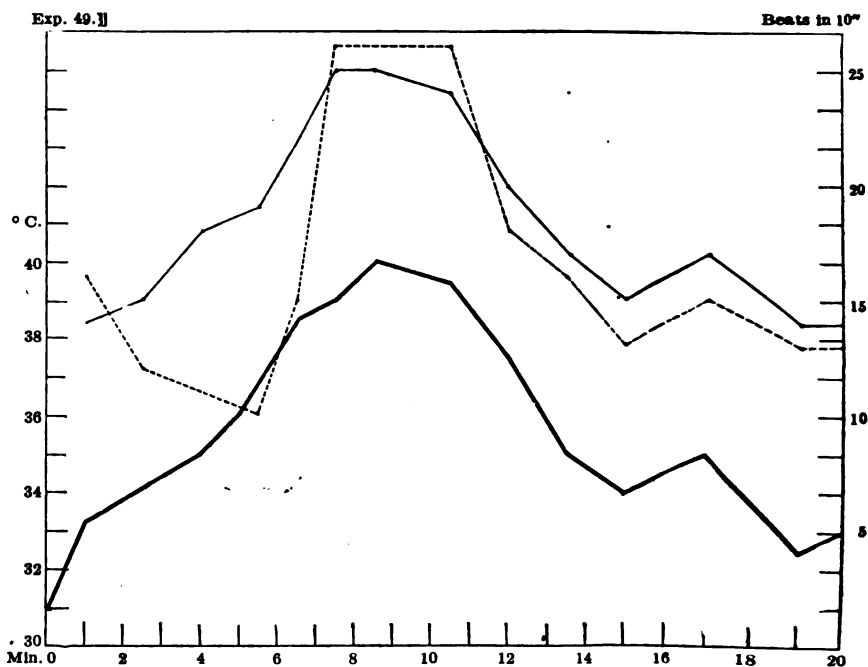


FIGURE 2.—The effect of changes of temperature upon the rate of the nodal strip — and septal strip ----. Temperature Abscissa: Time in minutes. Ordinates: Temperature in degrees centigrade, and number of beats in 10 seconds.

confirms the results of experimenters who maintain that the sino-auricular node is not a specific centre for impulse production, and shows that the coronary sinus is capable of responding to temperature changes and of regulating the rate of the heart. Flack² made some observations on the effect of cooling the sinus region, and described a slowing of auricular rate which could not be produced by cooling other parts of the superior vena cava. He also stated that after cooling the effect of the right vagus and accelerator nerves was abolished.

With isolated strips from the cat's auricle immersed in Ringer solution, we were able to study temperature effects readily. The accompanying curves (Figs. 2, 3) are examples of the results obtained. The

maximal degree of acceleration produced by warming was nearly equal in the nodal, coronary, and septal strips. The curve of acceleration of the nodal strip usually shows a sharper ascent in the early stages of

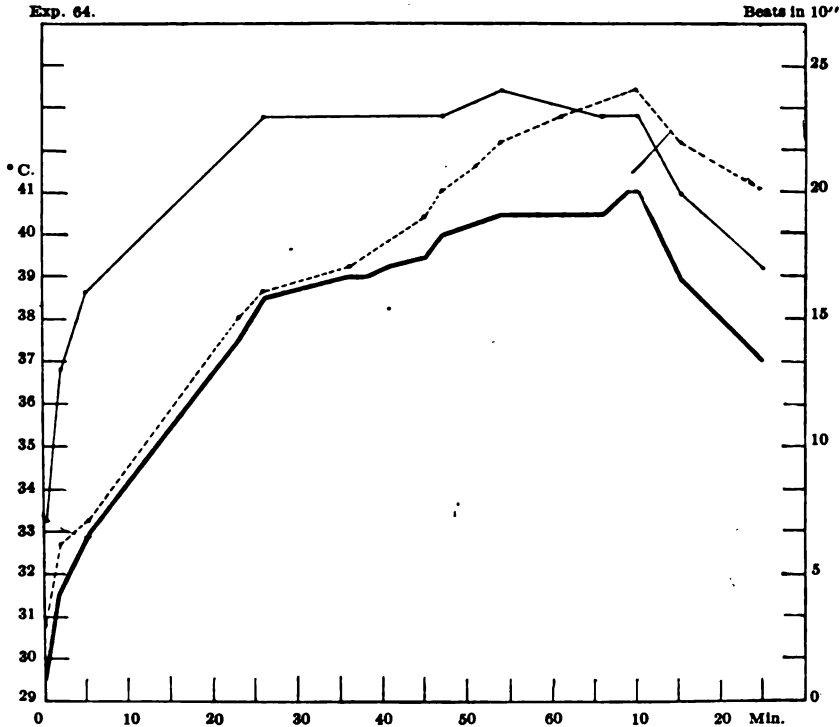


FIGURE 3.— The effect of changes of temperature upon the rate of the nodal strip — and coronary strip -----. Temperature Abscissa: Time in minutes. Ordinates: Temperature in degrees centigrade, and number of beats in 10 seconds.

heating than the curves of the other strips, as is shown in Fig. 3. Exceptionally the strips *B* and *C* showed a slowing of rate for an increase of temperature. This only occurred early in an experiment and disappeared in the course of warming (see Fig. 2), or gave way when a small dose of atropine was added to the bath. This initial slowing for increase of temperature was never observed with the sinus node strip, and only in two cases with the strips *B* and *C*. We are inclined to attribute it to increase in the irritability of intracardial inhibitory nerve cells under the influence of rapid warming, since it only appears early in an experiment and gives way to acceleration

when atropin is used. It will be seen from the curves that the strips which contained sinus tissue without specific tissue were capable of responding to increase of temperature with an increase of rate as great or even greater than the strip containing nodal tissue. Lowering of temperature produced a parallel decrease of rate in the strips used. The effect of cooling therefore may not be exerted on the nodal tissue alone, but on other areas of the sinus region equally. The strip *D* from the auricular wall showed an increase of rate for increase of temperature, but, having a lesser grade of rhythmicity than the other strips studied, showed a lesser power of reacting to rises of temperature.

Table II. contains the results of warming experiments. It will be seen that the degree of acceleration reached is approximately the same in the strips *A*, *B*, *C*. The percentage acceleration is generally higher in the septal and coronary strips.

Since Ganter and Zahn⁹ state that warming and cooling over the thickest portion of the node produced greater changes in rate than elsewhere along the course of the nodal tissue, temperature changes on strips so removed as to include these portions of nodal tissue were studied. We find that the effect of warming or cooling these strips produced parallel acceleration or slowing. This observation was made by Erlanger⁴ on his superior and inferior caval strips with the same result.

II. ELECTRICAL STIMULATION.

The results of this procedure were identical with those obtained with auricular strips of the dog's heart.⁵ The strips *A*, *B*, and *C* when stimulated and immersed showed an equal degree of acceleration.

III. DRUGS HAVING ACTION ON MUSCULAR TISSUE.

Caffeine. — Oswald Loeb's experiments¹ with the perfused hearts of cats and rabbits show that caffeine applied to the sinus region in 0.08 per cent solution caused a definite acceleration. When such a solution was applied to other parts, no effect was produced. With isolated strip preparations a well-marked acceleration is produced by 5 mgm. of caffeine in the 600 c.c. Ringer bath. Increase in rate, amplitude, and tone of the beat are observed. No marked differences

in the reaction of the nodal strip and the other strips from the sinus region were observed. Where the strips have become irregular in

TABLE II.

THE COMPARATIVE ACCELERATION OF THE VARIOUS STRIPS WITH RISE OF TEMPERATURE.

Expt. no.	Rate — Low temperature.		Temperature rise.	Time.	Rate — High temperature.		Percentage acceleration.	
	A.	B.			A.	B.	A.	B.
D 49	Beats per 10 seconds. 15	12	Centigrade. 34.0 → 40.0	Minutes. 6.0	Beats per 10 seconds. 25	26	Per cent. 66	Per cent. 112
50	14	10	32.0 → 39.0	12.0	21	19	50	90
51	18	16	34.0 → 38.5	10.0	20	25	11	56
Av.	15.6	12.6	22	23.3	41.0	84.0
Expt. no.	A.	C.	Temperature rise.	Time.	A.	C.	A.	C.
D 55	22	22	32.0 → 38.5	18.0	30	31	36.0	41.0
56	16	16	35.5 → 38.5	16.0	18	28	12.5	75.0
57	19	13	32.0 → 39.5	26.0	31	21	63.0	61.0
58	17	13	33.0 → 38.5	10.0	23	20	35.0	53.0
61	17	17	30.0 → 35.5	25.0	18	19	6.0	12.0
64	7	3	29.0 → 41.0	70.0	24	24	240.0	700.0
65	20	25	36.0 → 38.0	5.0	24	30	20.0	20.0
Av.	16.8	15.6	24.0	24.7	42.8	58.3
Expt. no.	A.	D.	Temperature rise.	Time.	A.	D.	A.	D.
60	15	6	33.0 → 40.0	20.0	25	9	66.0	50.0

action towards the end of an experiment caffeine has a distinctly beneficial action in improving the beat. The strip *D*, which exhibits a low grade of rhythmicity, shows a marked acceleration with caffeine.

Digitalis group. — The results obtained from treating auricular strips with drugs of this series are very like those described for the per-

fused heart. The strips in the Ringer bath to which one of the drugs has been added show an increase in tone and amplitude of beat, and in later stages an increase in the irritability of the tissue, terminat-

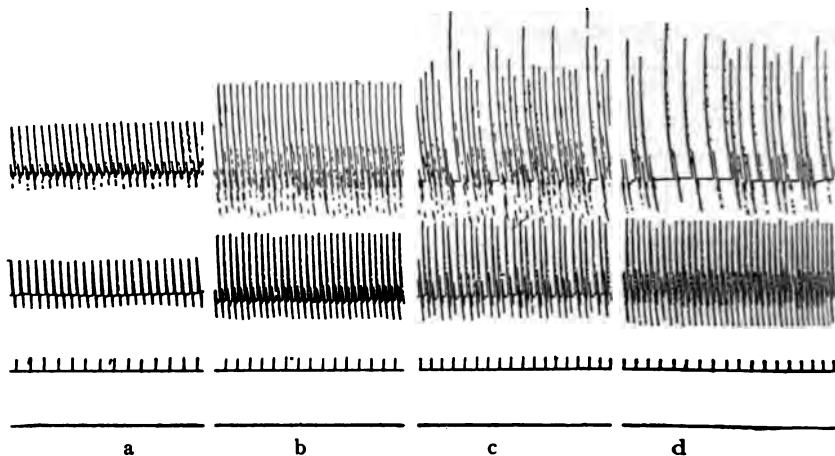


FIGURE 4.—The effect of strophanthin 5 mgm. The upper tracing is written by the nodal strip and the lower by the septal strip. Time in one-second intervals. *a*, normal; *b*, *c*, *d* are taken five, ten and twelve minutes respectively after treatment with strophanthin.

ing, if continued long enough, in fibrillation. The doses of the drugs which were found to produce the effect were:

Digalen, 1-2 c.c. ampoules, each c.c. = 0.3 mgm.

Digitoxin, 0.2 to 1 mgm.

Digitin, no effect observed with large doses.

Strophanthin, 2-4 mgm.

An example of the action of drugs of this group is seen in Fig. 4.

In two experiments with digalen an initial slowing was observed to occur in the strip *B*, while no such effect was seen on the nodal strip. It may be that we have here an example of the action of some of the digitalis group on the intracardial vagus mechanism which has been seen with the intact heart. This slowing was only temporary, and with further action of the drug gave way to acceleration and typical stages of digitalis poisoning.

Aconitin. — In two experiments using strips *A* and *B* the acceleration produced was greater in the strip *A*. Aconitin crystalline (Merck) was used in doses of 1 to 5 mgm.

In the few experiments done with this drug there seems to be evidence of a selective action on the nodal strip as compared with the nodal strip. The coronary sinus strip has not been tested with aconitin.

Chloroform. — Ringer solution saturated with chloroform was added to small amounts (1-5 c.cs.) with a resulting decrease in rate and

TABLE III.

EXP. 65. PROTOCOL SHOWING THE EFFECT OF CHLORAL ON THE RATE OF THE NODAL AND SEPTAL STRIPS.

Time in minutes.	Rate in ten seconds.	Rate in ten seconds.
	A.	C.
20.0	19	27
0.1 GM. CHLORAL HYDRATE.		
21.0	17	24
26.0	16	15
RINGER SOLUTION.		
30.0	18	20

size of beat. The various strips were equally depressed by the action of the drug.

Chloral hydrate. — Amounts of chloral hydrate up to 0.1 gm. produced a parallel depression of rhythmicity in the nodal, septal, and coronary strips. Table III. will illustrate this result.

The above results show that the properties of nodal, coronary, or septal sinus musculature are equally affected by various drugs having a beneficial or depressant action on cardiac muscle.

IV. DRUGS HAVING SELECTIVE ACTION ON NERVE ELEMENTS.

The most interesting differences in reaction of the strip containing nodal tissue and the other strips were observed with the drugs of this type.

Pilocarpine. — Flack² applied muscarine to the region of the sinus node and observed a slowing of the heart beat. In this initial stage stimulation of the vagus was greatly more effective. With longer applications of the drug the effect of vagus stimulation was abolished and the heart had a very slow rate.

Observations on the isolated strips brought out a most marked difference in the degree of action of pilocarpine on the nodal and other

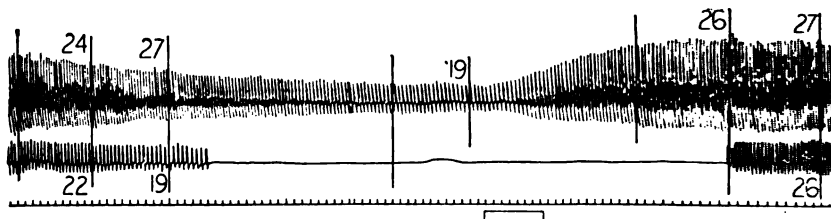


FIGURE 5. — Four sevenths the original size. Effect of pilocarpine and atropin. Upper tracing, contractions of nodal strip. Lower, septal strip. Pilocarpine 1 mgm. added five seconds before commencement of tracing. Atropine 1 mgm. given at signal mark.

strips. On addition of a dose of pilocarpine to the bath the nodal strip showed some decrease in height of contraction and in rate. The negative inotropic and chronotropic effects were far more marked with the strips *B* and *C*. As seen in the tracing (Fig. 5), these strips show a marked decrease in size and rate of beat, stopping altogether in a short period. This difference is evidently capable of explanation in one of two ways: either the vagal endings are less numerous in the nodal region, or their increase in irritability is more effectual in producing inhibition in the other areas.

This point can hardly be definitely settled as yet, but the fact remains that pilocarpine produces the most marked inhibitory effects on tissue which is free from specialized nodal musculature.

Physostigmine. — The effect of this drug was similar to that of pilocarpine, and the comparative action on the nodal and other strips from the sinus region was the same, — *i. e.*, a more marked inhibitory effect on tissue not containing the node.

Atropine. — Flack² shows that an application of atropine solution to the sinus region abolishes the effect of stimulation of the right vagus.

As shown in the tracing (Fig. 5), atropine counteracted the effect of pilocarpine or physostigmine. The beat of strip *A* improves mainly

in size, while *B* and *C* strips start beating vigorously. As was mentioned when we were dealing with temperature changes, the rate of the septal and coronary strips was twice slowed for an initial rise of temperature. Atropine had the effect of abolishing this slowing, and the rate of the strips was markedly accelerated for further rises of

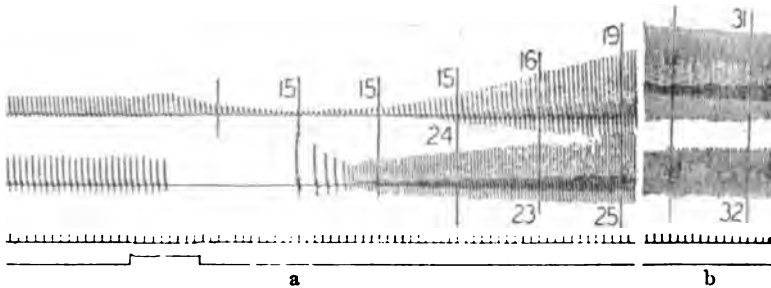


FIGURE 6. — Four sevenths the original size. Effect of nicotine upon nodal strip (upper tracing) and septal strip (lower tracing). Nicotine 5 mgm. at signal. *b*, three minutes later.

temperature. This indicated pretty clearly that the inhibitor mechanism was in a state of increased irritability due to the sudden rise of temperature. In one experiment the slowing of rate gave way to acceleration, without use of atropin, the strip evidently escaping from the inhibitor effect.

Nicotine. — Flack² observed that application of nicotine abolished the effect of the vagus on the heart. He makes no mention of changes of rate. Beyer,¹¹ working with perfused cats' hearts, has described all the changes taking place when nicotine is added to the perfusion fluid. There results an initial slowing followed by a marked acceleration. With further doses the period of slowing disappears, and the only effect produced is the acceleration.

The initial slowing produced by nicotine appeared just as described in Beyer's experiments. It was seen only early in the experiments and disappeared with successive doses and after atropin. Under these conditions nicotine produced acceleration. The conclusion that the initial effect is exerted on the intracardial ganglion cells is a pretty evident one. On the nodal strip this initial effect with small doses was mainly a negatively inotropic one, there being little or no decrease in rate. On both septal and coronary strips, however, the inhibitory effect was more marked, as is seen in the tracing (Fig. 6). These

¹¹ BEYER: Johns Hopkins Hospital reports, 1900, ix, p. 111.

strips usually stopped beating for a varying period and on resuming their rhythm rapidly developed a marked acceleration. The coronary strip was the one most markedly affected by initial inhibition. The secondary acceleration produced by nicotine has been explained by Wertheimer and Colas¹² as being due to an effect on sympathetic endings. In our experiments there was no marked difference observed in the degree or duration of the acceleration. If the strips were subjected to a more continued action of nicotine, irregularities and eventually fibrillation were produced.

As has been remarked in the case of pilocarpine, the difference in the initial effect of nicotine on the nodal and other strips may be explained either by a greater degree of innervation or a more effectual innervation of the tissue which is not nodal.

We have no evidence that the nerve endings of the vagus are less numerous in the nodal musculature than in the surrounding tissue. In the case of ganglion cells and presumably those belonging to the vagus apparatus, recent histological work may possibly throw some light on our results. Oppenheimer¹³ has lately described the nervous structures found in the sino-auricular node, and though he can demonstrate neurofibrils, has been unable to find any ganglion cells in the substance of the node in the dog. This observation points to the probability that the greater inhibitory effect produced on strips not containing the node by drugs having an action on ganglion cells is a result of quantitative differences in innervation. Marchand and Meyer³ applied nicotine solutions to the rabbit's heart to find out the chief areas of intracardial vagus innervation. They show in their diagram on p. 407³ that the region of the sinus overlying the inter-auricular septum and extending down over the junction of the left superior vena cava with the coronary sinus is the area where the action of the vagus can most readily be abolished. This region corresponds very well to the location of our septal and coronary strips, which have been shown to have a greater sensitivity to inhibitor influences.

In our experiments, it has been noted, the early stages of temperature increase may produce a slowing of rate in the septal and coronary strips. It has also been shown that drugs of the digitalis group

¹² WERTHEIMER and COLAS: Archives de physiologie, 1891, p. 341.

¹³ OPPENHEIMER, B. S., and ADELE OPPENHEIMER: Journal of experimental medicine, 1912, xvi, p. 613.

may in fresh preparations cause a slight slowing in the same strips. The drugs pilocarpine, physostigmine, and nicotine have also produced a stronger inhibitory effect on these areas. These observations, therefore, point to the probability that the vagus effect is mainly exerted on muscle tissue outside the node.

Epinephrin. — It is well known that epinephrin causes an increase in rate and output of the heart. Oliver and Schäfer¹⁴ have described the effect of suprarenal extracts on the intact mammalian heart. Gottlieb¹⁵ has demonstrated the great increase in rate and strengthening of the beat of the isolated mammalian heart under epinephrin. The effect produced by epinephrin on the isolated strip preparations was, as expected, a very marked increase in rate and amplitude of contraction and in tone. A distinct effect was observed with epinephrin dilutions of 1 to 240 millions, so that the strip preparations are fairly sensitive indicators of epinephrin. The increase in contractility is usually the earliest and sometimes the only effect observed. The acceleration occurs shortly after the strengthening of the beat and lasts for varying periods. The reactions of the nodal strip were compared with those of the other strips as to degree and duration of the epinephrin acceleration. The degree of acceleration reached was about the same for the nodal, coronary, and septal strips. Frequently the nodal strip showed a lesser degree of sensitivity to epinephrin. The acceleration lasted about the same time in the strips *A* and *C*, variations in favor of one or the other strip being small. The increase of rate of the strip *B* usually did not last as long as that produced in *A* or *C*. The auricular strip *D* usually gave a marked increase in rate lasting for short periods, but produced only by stronger solutions than those effective for *A*, *B*, and *C*. Strips from the ventricular wall gave a regular series of beats at a rate of 20 in ten seconds when treated with epinephrin solutions of 1 in 10 millions. These results are illustrated by the tracing (Fig. 7) published and the protocols in Table IV, which are selected from many similar observations.

Curara. — The use of this drug was suggested by Flack's observation² of the slowing effect produced by application to the sinus region. With the curara used in these experiments no effect was ob-

¹⁴ OLIVER and SCHÄFER: *Journal of physiology*, 1895, xviii, p. 230.

¹⁵ GOTTLIEB: *Archiv für experimentelle Pathologie und Pharmakologie*, 1897, xxxviii, p. 99; *Ibid.*, 1899, xliii, p. 286.

TABLE IV.

PROTOCOLS SHOWING THE COMPARATIVE DEGREE AND DURATION OF THE ACCELERATION PRODUCED BY EPINEPHRIN ON THE NODAL, SEPTAL, CORONARY, AND AURICULAR STRIPS.

Exp.	RATE.	A.	B.	Exp.	RATE.	A.	C.
32	Rate in 10'' Epinephrin 1- 30 mill.	18	18	58	Rate before 1-60 mill.	15	15
	Max. accel.	27	27		Max. rate	21	23
	Rate 3' later	24	21		Rate 2' after	17	18
40	Rate in 10'' 1-80 mill.	14	14	59	Rate before 1-120 mill.	17	22
	Max. rate	20	23		Max. rate	17	24
	Rate 3' after	14	14		Rate 5' later	16	21
53	Rate in 10'' 1-30 mill.	19	16	61	Rate before 1-120 mill.	16	17
	Max. rate	38	41		Max. rate	20	21
	Rate 1' later	31	31		2' later	16	17
	Rate 5' later	21	21	
46	Rate in 10'' 1-120 mill.	12	11	62	Rate before 1-120 mill.	14	16
	Max. rate	15	21		Max. rate	22	28
	Rate 1' later	14	17		after 2'	11	18
39	Rate before 1-150 mill.	19	16	61	Rate before 1-240 mill.	18	19
	Max rate	21	20		Max. rate	19	20
	Rate 1' later	19	20		2' later	18	18
Exp.	RATE.	A.	D.	Exp.	RATE.	A.	D.
60	Rate before 1-60 mill.	18	8	60	Max. rate 3' later	18	27
				17	5

served on the beat of any of the auricular strips, and there was no difference in response to pilocarpine or epinephrin after the curara had been used.

Apocodeine and ergotoxine. — These substances were used in the hope of depressing the sympathetic endings, and thus possibly bringing

out differences in the response to epinephrin and throwing some light on the accelerator innervation of the various strips. The results so far have been entirely negative. The accelerator endings are apparently very resistant to depressing effects.

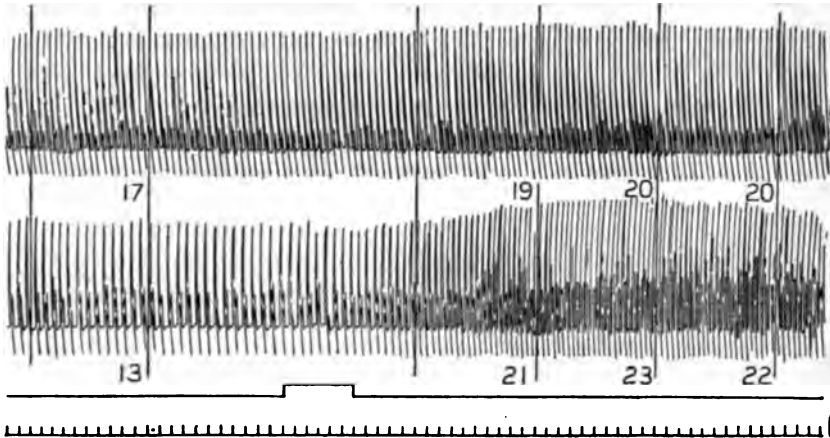


FIGURE 7. — Effect of epinephrin on nodal (upper tracing) and coronary (lower tracing) strips. Adrenalin chloride 1 to 120 millions at the signal.

Pituitary extract. — The use of this substance had little or no effect on the beat of the strips. Various preparations of the extract were tried with negative results.

SUMMARY AND CONCLUSIONS.

The experiments show that the isolated strips from the cat's auricle beat spontaneously in the Ringer bath. The rhythmicity of the coronary, nodal, and septal strips is approximately equal. Temperature changes do not affect the strip containing nodal tissue to a greater extent than the other strips from the sinus region. Variations in reaction of these strips to temperature have been noted. Drugs affecting cardiac muscle have been shown to exert a like effect on the three strips from the sinus region. The strip from the right auricle has a lesser degree of rhythmicity and reactivity. Drugs having an action on the vagus mechanism are consistently more effective on the strips not containing nodal musculature. Reasons have been given for believing that this depends on a quantitative difference in inner-

vation. Drugs having an action on sympathetic nerve endings produce an equal effect on the nodal, coronary, and septal strips, the acceleration being less lasting in the latter. The sino-auricular node in these experiments has not exhibited specially reactive properties to various influences affecting rhythm. The muscular tissues of varying character throughout the region of the mammalian heart related to the sinus venosus is possessed of equal powers of impulse production, as judged by its reactivity to the influences studied.

DIGESTION IN THE CHICK.

By T. P. SHAW.

[From the Department of Physiology, McGill University.]

INVESTIGATIONS upon the digestive functions of the chick have been mainly made with a view of determining what food stuffs produce the least digestive disturbance or result in the most rapid development.

The present investigation endeavors to throw light upon the physiological processes involved during digestion and thereby make the feeding of the chick less of an empirical process than at present.

Birds as a class stand in a lower evolutionary position than mammals. The absence of mastication, the crop, and the muscular organ the gizzard, are peculiar to the digestive apparatus of birds. The intestinal tract also shows peculiarities, especially in the double cœcum.

Considerable preliminary work had been done a year previous to the conducting of the present experiments to determine the best methods of investigating the digestive processes in the chick and to learn the technique. In the first preliminary experiment an adult hen was fed on a food containing starch and proteins (fat was not taken into account in this experiment). Six hours after feeding the hen was anæsthetized, the body opened, the various parts of the digestive tract tied off and removed. The experiment showed that sugar was present in the contents of the crop six hours after feeding. The contents of the proventriculus were acid in reaction and contained an enzyme which acts on protein, breaking it down to peptone. The gizzard grinds the hard grain to a soft sticky mass. When reduced to the proper consistency, it is moved along into the duodenum, where it comes into contact with bile from the liver and the juices of the pancreas and intestine. In the duodenum starches are hydrolized to maltose and dextrose, proteins to peptones, and peptones to amino-acids. The duodenum is slightly acid in the first part, alkaline in the

middle part, and in the last part the reaction is again acid, due to the presence of amino-acids and fatty acids.

METHODS.

My experiments are divided into two series. The first series of experiments were made with extracts of the floor of the mouth, crop, stomach (proventriculus), pancreas, and mucous membrane of small intestine. In the second series the chicks were fed on certain foods and the contents of the various parts of the digestive tract were examined for the products of digestion. The food given during these experiments was of known quality and was tested carefully to avoid errors. All experiments were conducted in duplicate, and the experiments themselves were repeated using different batches of chicks.

Method of preparing extracts. — The glandular structures in the floor of the mouth are so small it was found very difficult to dissect them out separately; so the lower jaw was removed, with the surrounding tissue, cut into small pieces and ground with sand in 1 per cent saline solution. A little toluol was added as a preservative. The extract of the crop was prepared in a similar manner.

Extracts of the stomach were prepared, using toluol water, pure glycerine, and glycerine and sodium carbonate. This last was prepared by adding 10 c.c. of a 1 per cent sodium carbonate solution to 90 c.c. glycerine.

Extracts of the pancreas were prepared with 1 per cent sodium carbonate solution and in pure glycerine.

A 1 per cent sodium fluoride solution was used for preparing extracts of the mucous membrane of the small intestine.

Chemical methods. — When a fair amount of material was available (a number of livers), Pflüger's method for the extraction of glycogen was used. When one or two livers only were to be examined, or when the livers could not be examined immediately, the presence or absence of dextrose in the liver extract after boiling with HCl was taken as indicating the presence or absence of glycogen.

Starch was tested for by the iodine test.

Fehling's test was used to show the presence of reducing sugar in the experiments on starch digestion.

To test for lactose in the rectum of chicks fed on milk the phenyl-

hydrazin test was used and the phenyl-lactosazone crystal prepared. Barfoed's solution was used to detect dextrose after feeding with lactose.

In the experiments for the detection of proteolytic enzymes fibrin was used, and the biuret (pink color) as showing the presence of peptone.

For the detection of lipase, milk or some neutral oil was placed in a test tube, a few drops of a 5 per cent sodium carbonate solution added until the reaction was alkaline to phenolphthalein, and 1 c.c. of the extract to be tested added. This tube with a control tube (in which the extract had been boiled) was placed on the water bath at a temperature of 40° C. The disappearance of the pink color was taken as positive of the presence of lipase.

General. — In all the experiments the extracts were made from living tissue. The chick was opened immediately after dislocation of the neck and the organs removed before the heart had stopped beating. The extracts were allowed to stand a reasonable time after being prepared. Toluol was used in all experiments where bacterial action might be a factor.

All experiments in test tubes were kept at a temperature of 40° C. for two hours before final results were recorded.

The extract of small intestine in sodium fluoride was incubated at 40° C. for twenty-four hours before testing. This last experiment was not satisfactory.

EXPERIMENTS WITH EXTRACTS.

1. Extract of floor of mouth. —

Age of chick.	Effect on starch paste.	Remarks.
One hour after hatching.	Hydrolyzed to sugar in less than half an hour.	No effect on protein or fat.
Twenty-four h'rs old.	"	"
Seventeen days old.	"	"

The glands in the floor of the mouth of the chick secrete an amylolytic ferment, present from birth. This ferment acts in an alkaline medium.

2. Extract of crop. —

Age of chick.	Results.
One hour old.	No effect on starch paste, protein, or fat.
Twenty-four hours old.	" "
Seventeen days old.	" "
Twenty-four days old.	" "

The crop does not secrete any enzymes which act on starch paste, protein, or fat. Microscopic section shows that it contains mucous glands. The crop measures about five sixteenths of an inch in diameter at birth.

3. Extract of stomach. —

Age of chick.	Action on starch paste.	Action on protein.	Action on fat.
Two days.	Negative.	Peptone present.	Negative.
Seventeen days.	"	"	"

The stomach is acid in reaction to litmus. Within two days of hatching a watery and a glycerine extract were obtained, which digested fibrin to peptone in the presence of 0.2 per cent HCl. Negative results were obtained in our endeavor to demonstrate the presence of a curdling ferment or a lipolytic ferment in the extract.

4. Extract of pancreas. —

Age of chick.	Action on starch paste.	Action on protein.	Action on fat.
Two days.	Erythro-dextrin present, no sugar.	Peptone present.	Negative.
Seventeen days.	Sugar present.	"	Fatty acids present.

The extract acted best in a slightly alkaline medium, and the presence of bile seems to hasten the action.

The pancreas is a small thread-like structure, at birth lying in the loop of the duodenum. The three pancreatic ducts open near the termination of the duodenum (end of distal limb) beside the two bile ducts.

EXPERIMENTS WITH THE CONTENTS OF THE DIGESTIVE TRACT.

1. Examination of contents of crop after feeding food containing starch.—

Age of chick.	Material fed.	Time.	Result.	Remarks.
Eleven days.	Egg and starch.	Two hours.	Starch and sugar present.	Alkaline to litmus.
Twenty days.	Chick food, free from sugar.	Four hours.	"	"

The starch grains swell up under the influence of moisture, causing the cellulose coat to burst and allowing the ferment present in the saliva (First series, Exp. 1) to act on the starch. Even though the food is retained in the crop for several hours all the starch is not acted on, as starch was found in the stomach and duodenum.

Bacteria may be a factor in breaking down the cellulose coat. No ferment acting on cellulose was found.

2. The contents of the crop after feeding milk. — Chick, two days old, fed on milk, crop examined after two hours.

No peptone reaction was present, contents fluid without flocculi or curds. Fehling's solution was reduced by the milk sugar present.

3. Examination of contents of proventriculus. — (a) Stomach of chick, examined twenty-four hours after hatching, found to contain a yellowish, watery fluid which gave no peptone reaction.

(b) Age of chick.	Material fed.	Time.	Results.
Two days.	Egg and starch.	Two hours.	Peptone, starch, and sugar present.
Four days.	Milk.	Six hours.	Peptone, sugar, and small curds.

Soon after birth the stomach of the chick is able to perform its digestive functions and secretes proteolytic and curdling ferments.

Other investigators have found pepsin and a curdling ferment in the stomach of birds, but all previous experiments have been conducted upon adult birds.

4. The contents of the duodenum. —

Age of chick.	Material fed.	Time.	Results.	Remarks.
Two days.	Milk.	Two hours.	Peptone and lactose.	No glycogen in liver.
Four days.	Egg and starch.	Peptone and starch present, no sugar.	Liver contained glycogen, sugar in crop and stomach.
Seven days.	"	Sugar, starch, and peptone present.	"

In the contents of the duodenum of a chick two days old we find peptone and lactose, but no glycogen in the liver. At four days old the functions of the pancreas are still undeveloped, and the glycogen found in the liver was probably formed from the sugar absorbed from the digestive tract above the duodenum.

The peptone found in the duodenum on the fourth day may or may not have been produced by the action of the pancreatic juice. Exp. 4 of the first series of experiments shows that an active proteolytic ferment can be extracted from the pancreas on the second day; still we are unable to exclude in this experiment the possibility of the peptone found having been produced in the stomach. By the seventh day after birth the functions of the pancreas are well developed.

5. OBSERVATIONS ON THE LIVER.

Age.	Results.	Remarks.
(a) Twentieth day of incubation.	Glycogen present.	Livers of four chicks taken.
Twenty-four hours old.	Glycogen absent.	Two chicks taken; no food taken.
Two days old.	Glycogen present.	Chicks fed on egg and starch.
Four days old.	Glycogen present.	Chicks fed on egg and starch.
(b) Twenty-four hours old.	Glycogen absent.	Two chicks taken, fed on milk, two hours before killing.
Three days old.	Glycogen absent.	Six chicks fed on milk since birth.

Glycogen in the foetal liver. — The chick has no placental attachment during gestation and must draw its nourishment from the food material with which it is surrounded, which contains a very small amount of carbohydrate.

Our experiments show that not only is lactose not digested, but that it acts as an irritant to the gastro-intestinal mucosa.

Six chicks fed from birth on milk alone died on the third day, and the mucous membrane of the stomach and intestine was found intensely inflamed, with numerous hæmorrhagic areas scattered over the surface. Barfoed's reagent was not reduced by the contents in any part of the intestinal tract. Lactose was proved to be present by preparing phenyl-lactosazone crystals.

PTYALIN IN THE SALIVA OF THE CHICK.

Ptyalin has been found in the saliva of most animals. Investigators working on digestion in birds do not mention having discovered ptyalin in the fowl. No previous work, as far as we know, has been done to demonstrate its presence in the chick.

Our experiments show that the saliva is the only digestive juice capable of acting on starch for the first few days after the chick leaves the shell. In its struggle to free itself from the egg the chick uses up most of the glycogen stored in the liver. For the next twenty-four hours it naturally remains quiet, hunger asserts itself, and the chick looks for food. Starch given at this time is in part hydrolyzed by the ptyalin of the saliva while in the crop. The sugar thus formed is quickly absorbed.

Proteolytic ferments are present in the stomach and pancreas soon after birth, the other ferments of the pancreas are developed several days later.

We endeavored to demonstrate the presence of a ferment which would act on cellulose, but were unable to do so. Schottelius¹ has shown that the presence of bacteria is an important factor in the digestion of cellulose in chickens.

Nothing has been said in regard to the function of the gizzard in this paper, for the reason that there is considerable controversy going on at present as to whether grit is necessary for the gizzard function.

¹ SCHOTTELIUS: *Archiv für Hygiene*, 1899, **xxxiv**, p. 210; 1902, **xlii**, p. 48.

From our observations we would consider that it was necessary, but do not wish to make any very positive statement until we have investigated the matter further.

SUMMARY.

1. Extracts of the glandular structures of the floor of the mouth contain amylolytic ferment active in an alkaline medium. This ferment was found in extracts made from a chick one hour after hatching.

2. The crop secretes no ferment. The crop acts as a digestive organ by retaining the food for a considerable time, thereby allowing the ptyalin in the saliva to act on the starch content.

3. By the second day the stomach of the chick secretes a gastric juice which contains proteolytic and curdling ferments active in acid medium.

4. The pancreatic secretion in the chick contains proteolytic, amylolytic, and lipolytic ferments which act best in a slightly alkaline medium. The functions of the pancreas are imperfectly developed before the seventh day after birth.

5. The liver of the chick contains glycogen on the twentieth day of incubation. It becomes glycogen-free twenty-four hours after hatching if no food has been given. Glycogen was found in the liver on the second day after food containing starch had been given.

6. Lactose is not a glycogen former in chicks and acts as an irritant to the gastro-intestinal mucosa.

The author wishes to express his thanks to all those who have assisted him in any way, and he is especially grateful to Professor Alcock for his valuable suggestions and advice. He has used freely the valuable review of the literature on digestion contained in Bulletin No. 56 of the United States Department of Agriculture, by E. W. Brown.

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**PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL
SOCIETY.**

TWENTY-FIFTH ANNUAL MEETING.

WESTERN RESERVE UNIVERSITY, DECEMBER 29, 1912 TO JANUARY 1, 1913.



PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.

A NEW FORM OF SPLEEN ONCOMETER.

By D. E. JACKSON.

THE oncometer is made of sheet brass nickel plated. The length is 12 cm., width 4 cm. at the top by 4.5 at the bottom. The greatest depth is 2.8 cm. The lid slides in and out, and the mesentery of the spleen fits into the groove in the lid. The tip of the spleen fits into the end of the oncometer to which the spout is attached. Rubber tubing passes from the spout to the recording tambour. The whole oncometer with the enclosed spleen is placed in the upper left part of the abdominal cavity, the intestines are fitted around the instrument, and the abdomen is closed. No vaseline is needed.

HEMATOGENOUS JAUNDICE AND ITS RELATION TO THE LIVER.

By G. H. WHIPPLE.

THE object of this communication is to point out that hemoglobin can be transformed into bile pigment in the circulation when the liver has been excluded from any participation in this reaction. When hemoglobin is given intravenously in a normal dog, it will appear in the urine in ten to fifteen minutes, followed by bile pigments in one to two hours (Huppert's test). After the production of an Eck fistula, this same procedure will give identical results. When an Eck fistula is

produced and the hepatic artery is ligated, the injection of hemoglobin will be followed by the appearance of hemoglobin in the urine in ten to fifteen minutes and bile pigment in one and a half to two hours (death, five to six hours). An experiment in which the aorta below the subclavian is clamped, ligatures placed on the aorta at the celiac axis and bifurcation, on the vessels in the gastro-hepatic omentum and on the mammaries, will show after injection of hemoglobin a beautiful jaundice coloring of the fat on the thorax and in the mediastinum. Bile pigments can be demonstrated in this fat and plasma, while below the diaphragm the fat and plasma are negative (death, four to five hours).

Experiments in which the circulation is limited to the head and thorax by means of clamps on the aorta below the subclavian, ligatures on the mammaries, along the costal margin and on the vena cava above the diaphragm, show exactly similar results. With a vigorous circulation under these conditions for two hours there can be a transformation of hemoglobin into bile pigments. We may assume that the liver furnishes a ferment, present in the blood stream, which is capable of effecting this transformation even after the liver is cut out of the circulation. Negative experiments *in vitro* with serum, hemoglobin, and tissue extracts speak against this explanation. A more probable explanation, we believe, is that the endothelial cells of the organs and tissues, aside from the liver and spleen, can transform the hemoglobin in the circulation into bile pigments.

ON THE EXCRETION OF NITROGEN SUBSEQUENT TO
LIGATION OF SUCCESSIVE BRANCHES OF THE RENAL
ARTERIES.

BY J. D. PILCHER.

THE observations were made on one cat and one dog. The total nitrogen excretion was determined. Ligation of one of the branches of both renal arteries, *i. e.*, approximately half of the blood supply, does not cause any noticeable disturbance. The urine and the nitrogen excretion remain practically normal, with a slight tendency to nitrogen retention, which is probably not accidental. Successive com-

plete ligation of one entire renal artery and one branch of the other — *i. e.*, eliminating about three fourths of the arterial supply — causes marked temporary prostration, anorexia, and loss of weight, with the nitrogen output considerably greater than the intake. The animals recover gradually to a condition similar to that when but one half of the arterial supply was ligated. On the assumption (justified by Mac-Nider's histological data) that the ligated areas took no part in urine formation, the remaining one fourth of the kidney was able to secrete urine almost as effectively as the entire kidney substance.

The quantity of urine did not vary. With three fourths of the arterial supply ligated the urine contained no albumen nor casts of any description.

Occlusion of one branch of the renal artery does not result in sufficient collateral circulation from the capsule to preserve the function of the ligated area; so that even after ten months it is unable to preserve life if the other branch of the renal artery is then ligated.

FURTHER STUDIES ON THE RÔLE OF THE HYPOPHYSIS IN THE METABOLISM OF CARBOHYDRATES — THE AUTONOMIC CONTROL OF THE PITUITARY GLAND.

BY LEWIS H. WEED, HARVEY CUSHING, AND CONRAD JACOBSON.

IN a former paper from the Hunterian Laboratory in Baltimore it was stated that "in view of the ease with which hyperglycæmia may be produced by hypophyseal lesions, it is possible that our views in regard to the glycosurias of supposedly encephalic origin need some revision." Studies pursued in this direction have led to the following observations:

Provided there is a storage of glycogen available for discharge, —

1. A piqûre of the hypophysis in the rabbit is comparable, in its glycosuric response, to a piqûre of Bernard's so-called sugar centre in the fourth ventricle.
2. Stimulation of the superior cervical ganglion, by faradization or even by the manipulations necessary for its exposure, causes glycosuria in the rabbit, cat, and dog.

3. Stimulation of the superior cervical ganglion, after exclusion of all possible downward impulses to the abdominal viscera by way of the vagi, cervical sympathetic trunks, or spinal cord, leads to glycosuria.

4. Stimulation of the superior cervical ganglion, after separation of all synapses of the sympathetic system by administration of nicotine, causes glycosuria.

5. Direct faradic stimulation of the hypophysis itself, after exposure by a transphenoidal operation, gives glycosuria, even after preliminary transection of the spinal cord and cervical sympathetic trunks.

6. If the posterior lobe of the hypophysis has previously been removed by operation, the usual stimulation of the superior cervical ganglion fails to give glycosuria.

7. Direct faradic stimulation of the hypophysis provokes glycosuria, even after transection of the spinal cord above the splanchnics.

8. A Bernard piqure will likewise cause glycosuria, even after transection of the spinal cord above the splanchnics.

CONCLUSIONS.

The pituitary body, and more particularly its posterior lobe, plays a significant rôle in the metabolism of carbohydrates, and its action in this respect is under the control of fibres which reach the gland by way of the superior cervical sympathetic ganglion. Stimulation of this nervous pathway at the so-called sugar centre in the fourth ventricle, at the superior cervical ganglion, and by excitation of the pituitary body itself, liberates a chemical substance which causes glycogenolysis and glycosuria, independent of any possible nervous impulse reaching the glycogen-holding cells of the muscles or abdominal viscera.

THE EFFECTS OF AORTIC COMPRESSION ON THE CIRCULATION.

BY TORALD SOLLMANN AND J. D. PILCHER.

I. *Vasomotor centre.* — By the use of our perfusion method we have shown that the rise of blood pressure on compression of the aorta is normally accompanied by a moderate constrictor response of the

vasomotor centre, presumably due to the increase of intracranial pressure. No evidence of depressor effect could be found; it is possible that depressor stimulation occurs, but that this cannot make itself felt against the more powerful constrictor impulses. The depressor mechanism therefore does not protect the heart against excessive pressures, whatever may be its function.

II. *Influence of the level of blood pressure.* — If the vagi have been cut, aortic compression gives the same *absolute* rise, for all levels of blood pressure between 40 and 120 mm. The average *percentile* rise therefore varies inversely to the level of blood pressure. This corresponds exactly to the pressure response to sciatic stimulation. The percentile rise is therefore not a reliable index of vasomotor response.

III. *The release fall.* — When the aorta is released, the pressure falls momentarily below the normal. This is not due to vasomotor paralysis (as generally assumed), but to lessened cardiac efficiency. The heart is not injured directly by the compression, but the return flow of blood is temporarily lessened by the release of the aorta, and the tone of the cardiac muscle requires a little time to readjust itself to the altered pressure conditions.

THE SURFACE TICKLE SENSE OF THE HUMAN SKIN.

BY WARREN P. LOMBARD.

THE object of these experiments was to determine whether the pressure and tickle sensations, called out by delicate mechanical stimuli, arise from the same spots. The experiments were made by the writer on himself, and chiefly on an area 5 mm. square on the back of the hand. Concordant results were obtained only after the skin had been thoroughly softened with vaseline, and the excitations were given while the skin was being watched through a Zeiss binocular microscope magnifying ten times. A soot print showing the grooves in the skin was enlarged photographically ten times, and gave an accurate map, upon which the position of the spots was recorded. The area was found to contain 5 pressure, 14 tickle, 3 cold, no warmth, and a

very great number of pain spots. The position of 16 pressure and tickle spots was entered on a map in 1910; and one year later, without reference to the old map, 14 of these points were relocated within 0.2 to 0.4 mm. of their former positions. The stimuli were given by Von Frey's "Reizhaare," and by the weight of fine needles, glass rods, and bristles, which slid with almost no friction in two holes in a little guide frame. The results did not depend on the mental attitude of the subject, because pressure and tickle spots were often properly located where different sensations were expected. The final test is the striking difference in the after sensations. The threshold for tickle spots is only slightly lower than for pressure spots, and in both cases is largely a function of the flexibility of the skin. A bristle rod having an end diameter of 0.30×0.24 mm., and weighing 0.003 gm. may cause a distinct tickle sensation. The irritability of tickle spots is more variable than that of pressure spots, and they are more subject to fatigue. Many of the peculiarities of the tickle sense are probably of central rather than peripheral origin; namely, the increasing irritability resulting from excitation, the fact that exciting one point may raise the irritability of neighboring points, irradiation phenomena and consequent imperfect localization of the sensation, the prolonged after tickling which passes over into itching and excites reflexes, summation effects, and the inhibition of tickling caused by strong mechanical excitations. This inhibition does not come from the superficial nerve endings which give surface tickle and surface pressure sensations, but from deeper nervous structures. The pressure sense shows many of these peculiarities, but to a much less degree.

FEEDING EXPERIMENTS RELATING TO THE NUTRITIVE VALUE OF THE PROTEINS OF MAIZE.

BY THOMAS B. OSBORNE AND LAFAYETTE B. MENDEL.

ACCORDING to the data now available more than one half of the proteins of maize consists of zein, a type exhibiting such unique chemical and physical characters as to make probable that its nutritive

properties differ from those of other proteins. About one third of the proteins consists of maize glutelin, insoluble in neutral solvents, extracted from the seed only by dilute alkalies, and yielding all the amino-acids characteristic for most other proteins. When zein forms the sole protein of the dietary, rats speedily decline in weight despite an apparently sufficient food intake. The decline is not due to digestive failure; for the food can be made adequate for maintenance over a considerable period by the addition to it of tryptophane (which is missing among the decomposition products of zein). When half of the zein is replaced by another protein, such as casein, lactalbumin, edestin, or maize glutelin, nutritive decline can be checked. The proportion necessary varies with the different proteins. In contrast with zein, which lacks tryptophane, lysine, and glycocoll, gliadin, which is deficient in the last two only, suffices for maintenance without growth. Maize glutelin is adequate for normal growth. Foods containing equal parts of zein and maize glutelin promote nearly normal rate of growth; this applies likewise to the natural mixture of them as exhibited in so-called corn gluten. This material affords an opportunity to study the nutritive value of the two maize proteins before they have been subjected to any chemical manipulations. Animals kept on foods containing additions of both tryptophane and lysine to zein have been maintained over long periods of time. These observations all emphasize the extreme importance of tryptophane in successful dietaries. It also appears probable that the deficiency observed in the practical feeding of corn meal is explained in good part by the unique chemical constitution of zein, which forms so large a part of its nitrogenous components.

PERFUSION OF THE RESPIRATORY CENTRE IN FROGS —
THE INFLUENCE OF CALCIUM AND POTASSIUM ON THE
RESPIRATORY RHYTHM.

BY D. R. HOOKER.

THE fore-brain was transected, the abdominal wall opened, and the perfusion cannula tied into the bulbus. The throat muscles were used to record respiratory rhythm. The normal solution contained NaCl

0.7, urea 0.2, dextrose 0.1, KCl 0.03, and CaCl₂ 0.03 per cent; it was fed at a pressure of about 30 cm. under an oxygen atmosphere. Under these conditions the respiratory movements continued for four hours or more; they ceased upon destruction of the medulla, and were influenced by the rate of perfusion and by the presence of carbon dioxide.

In the absence of calcium the centre was excited, and in the absence of potassium it was depressed. In the presence of potassium a decrease in the calcium caused depression, and an increase caused excitation. In the presence of calcium a decrease in the potassium caused excitation, and an increase caused depression.

CARDIAC SERUM ANAPHYLAXIS IN THE RABBIT AS SHOWN BY THE STRING GALVANOMETER.

BY G. CANBY ROBINSON AND J. AUER.

YOUNG rabbits were sensitized with repeated subcutaneous and intraperitoneal injections of horse serum. About four weeks after the last injection the rabbits were prepared for recording their electrocardiograms. The right front leg and the left hind leg (lead II) were usually employed, and the records made with the large Edelmann instrument. No anæsthetics were used, the final or toxic injection being given through the marginal ear vein. The electrocardiograms of seven rabbits, in which the toxic injection proved fatal, show that disturbances of the heart beat usually develop swiftly, often before the end of the serum injection, at a time when the respiration is fully adequate. The most striking disturbance is partial dissociation between auricles and ventricles, which may increase and become complete. Usually the normal sequential beat is re-established, and the dissociation may occur and clear up several times. Finally, however, a period arrives in highly sensitized rabbits when the process or processes occurring in the heart are no longer reversible, and the record then shows a slow heart rate with ventricular and auricular arrhythmias and dissociations.

The shape of the ventricular portions of the electrocardiograms often show changes shortly after injection which are very similar to

those obtained after respiration has ceased: slow descent of the R wave, with the enlarged T wave very close to the R wave, which seem to be characteristic of a dying heart in general, as shown by one of us (Robinson). These early changes in the form of the electrocardiogram may be temporary, and be followed by electrocardiograms practically normal in form.

The conduction time between auricles and ventricles often shows a series of oscillations between a normal, a decreased, and an increased conduction interval which would indicate that some reversible process is taking place in the structures regulating the mechanism of the heart beat.

This report deals only with the experiments in which the final injection of horse serum caused death.

THE EFFECT OF STRYCHNIN IN CARDIECTOMIZED FROGS WITH DESTROYED LYMPH HEARTS.

BY S. J. MELTZER.

IN several communications we have reported that the injection of solutions of strychnin, morphin, or acid fuchsin in cardiectomized frogs is liable to bring on convulsions of these animals. The lymph hearts continue to beat for a while after the cardiectomy. But since the lymph hearts assist the circulation only by emptying their contents into veins, it seemed to be evident that the removal of the blood heart eliminates also the circulatory function of the lymph hearts. I have therefore supposed that the above-mentioned alkaloids reach the central nervous system by way of the lymph spaces which are connected throughout the body and which are capable of serving as a path for distribution by means of a peripheral mechanism. In a recent paper by Abel,¹ in which our facts were confirmed and in which it was admitted that the activity of the posterior lymph hearts cannot come into consideration, the statement was made that "the appearance of convulsions in the experiments of Meltzer and his pupils with acid fuchsin, morphin, and strychnin depends entirely on the integrity

¹ ABEL: *Journal of pharmacology*, 1912, iii, 581.

of the anterior lymph hearts." This statement is supported by a report of experiments in which, after destruction of the anterior lymph hearts in addition to cardiectomy, the alkaloids under discussion did not bring on any convulsions. I shall not enter here into a discussion of the entire subject. I merely wish to say that, at least for strychnin, it is very easy to show by the following simple experiment that a sufficient dose will bring out convulsions or tetanus in frogs after removal of the blood heart and destruction of the anterior lymph hearts. Under ether anæsthesia the entire thoraco-abdominal viscera, including heart and large blood vessels, is removed, the blood wiped out, and the anterior lymph hearts, which are now readily visible, are destroyed. When now 10 mgm. or more of strychnin are injected into each femoral lymph sac, a tetanus will appear after thirty to fifty minutes, even after this, most destructive operation, provided the animals are kept at low temperature. Abel's failure to obtain results may have been due to his experimenting during the spring. We indicated in previous communications that morphin failed to produce an effect upon cardiectomized frogs even in April, and we could obtain hardly any effect with acid fuchsin in the latter part of May.

THE RELATION OF FATIGUE METABOLITES TO EPINEPHRIN EFFICIENCY.

By R. G. Hoskins.

KRETSCHMER¹ has reported that the action of epinephrin is enhanced by the simultaneous injection of acid. There is evidence² that an augmented discharge of epinephrin from the adrenal glands occurs during periods of strong emotion, sensory stimulation, and asphyxia — conditions normally associated with extensive muscular activity. Such observations suggest that acid metabolites might adaptively increase epinephrin efficiency. The possibility was investigated in dogs by noting the effects of a constant dose of adrenalin under various ex-

¹ KRETSCHMER: *Archiv für experimentelle Pathologie und Pharmakologie*, 1907, lvii, p. 423.

² CANNON and DE LA PAZ: *this Journal*, 1911, xxviii, p. 64; CANNON and HOSKINS: *Ibid.*, 1911, xxix, p. 274; ELLIOTT: *Journal of physiology*, 1912, xlv, p. 374.

perimental conditions. Blood pressure was used as a criterion of the activity of the drug. That quantity was employed in each case which under normal conditions gave a rise of about 20 mm. Morphine-urethane anæsthesia was used. The effects of asphyxia due to breathing through a six-foot tube, of stimulating the peripheral end of the cut sciatic nerve, of injecting lactic acid (10 to 30 c.c. 5 per cent), of monopotassium acid phosphate (10 to 40 c.c. 5 per cent solution) and of convulsive dosage of strychnine were investigated. In each case there occurred an augmentation of respiration, showing that an effective dosage had been used. There was, however, no perceptible increase in the effect of the standard dose of adrenalin.

THE SPLANCHNICS AS A DEPRESSOR NERVE.

BY J. AUER AND S. J. MELTZER.

IN disagreement with the accepted view we found in a great majority of experiments on dogs that electrical stimulation of the central end of the splanchnic nerve causes an unmistakable drop in blood pressure. Both vagi were cut; the splanchnics were stimulated either in its course under the diaphragm or in the thorax below the tenth rib. It required a fairly strong stimulus to cause a drop; practically in no case and with no stimulus was a rise effected. The drop amounted in many instances to 50 or 60 mm. of mercury and in some cases even to more. The pressure could be held down evenly for half a minute and longer in the first stimulation; in the second stimulation the drop was less deep, and in the third still less. However, after an interval of rest, responsiveness was fully restored. Only few experiments were made with both splanchnics cut; under these conditions it seems that the ensuing depression may even lead to a fatal termination. Very few experiments were made on cats and rabbits. A rise occurred in cats; in rabbits, however, a short stimulus gave a moderate but unmistakable fall of the blood pressure.

The opposite results obtained forty-four years ago by Asp and Ludwig may perhaps be explained by their failure to use an anæsthetic in addition to curare. Our animals were well anæsthetized by ether,

which eliminated the action of the sensory fibres contained in the splanchnic nerves.

The abdominal viscera, then, like the viscera of the thoracic cavity, have their own reflex depressor nerve. Furthermore, since the effects of a vasoconstriction in the periphery of the splanchnic nerves may act as a stimulus upon the reflex depressor nerve and thus automatically check the excess of constriction, the presence in the splanchnic nerves of two kinds of fibres, antagonistic to one another, one causing directly peripheral vasoconstriction, and the other causing reflexly vasodilatation, may signify a self-regulating mechanism.

CHEMICAL CHANGES IN THE NERVE FIBRE DURING PASSAGE OF NERVE IMPULSE.

BY SHIRO TASHIRO.

1. I HAVE constructed an apparatus which not only detects carbon dioxide in quantities as small as 0.0000001 gm., but estimates it with quantitative accuracy.

2. With this apparatus I have established the following facts:

(a) The resting nerve fibre, both medullated and non-medullated, motor and sensory, gives off CO₂. This was established for invertebrates and vertebrates, both warm-blooded and cold-blooded.

3. The quantitative determinations are as follows:

1. Non-medullated fibre of spider crab 6.7×10^{-7} gm. per 10 mgm. per ten minutes.

2. Medullated fibre of frog 5.5×10^{-7} gm. for the same units.

(b) The nerve fibre gives off more CO₂ when stimulated by electrical, chemical, thermal, and mechanical stimulation.

The increase amounts to about 2.5 times, as shown in

1. Non-medullated fibre of spider crab 16.0×10^{-7} gm. for the same units.

2. Medullated fibre of frog 13.6×10^{-7} gm. for the same units.

(c) The CO₂ production from the resting nerve is altered according to changes of excitability brought about by long standing, ether, KCl, and killing.

3. From these facts the following conclusions are reached:

(a) In spite of all the negative evidences so far advanced we have established that the nerve fibre has a metabolism, and that this metabolism is directly connected with state of excitability.

(b) The increased CO_2 production by stimulation can be used as a new criterion for protoplasmic excitability. Thus it serves as one of the methods by which we can detect the vitality of protoplasm. The preliminary tests on dry seed and other plant and animal tissues confirmed the usefulness of this new criterion.

STUDIES IN EXPERIMENTAL CRETINISM, WITH SUGGESTIONS AS TO A BIOLOGICAL TEST FOR THYROID SECRETION.

BY ARTHUR L. TATUM.

A SYSTEMATIC study of the microscopic anatomy of cretin rabbits has brought to light some new relationships, also a partial confirmation and extension of the findings of Hofmeister, Benson, Kishi, Langhans, and others.

Ascites, excessive pericardial and synovial fluids, and tissue edema are constantly found. The heart muscle cells are swollen, and are generally represented by a single external layer of sarcostyles. The liver has fatty degeneration in the mid and central zones on the lobules, with often a great swelling of cells due to serous imbibition. The kidney shows fatty and granular degeneration of the cortical cells, and hydropic imbibition of cells in the collecting tubules and ducts of Bellini. The lungs show atelectasis, bronchiectasis, and emphysema. The ovaries and testes are degenerate and undeveloped. The hypophysis and parathyroid present a cellular swelling, together with serous and fatty vacuolation. Neither acini nor colloid is found in either organ. In the pancreas the islands of Langerhans are hypertrophied and hyperplastic. The thymus shows hypertrophic Hassels corpuscles and atropic lymphoid elements. Smooth and voluntary muscle exhibit serous infiltration and fatty degeneration, which may account for the "pot belly" symptom of all cretins. Bone marrow is of - " with leucocyte centres (Bunting)

in excess of the erythrocyte centres. The adrenals have enlarged medullas, with excessively fat cortices.

An attempt is being made to use the cretin rabbit as a test object for the detection of thyroid secretion in blood or serum. This line of work is being continued and will be reported on in detail at its completion.

THE EFFECT OF THYROIDECTOMY AND CASTRATION, RESPECTIVELY, ON THE WEIGHT OF THE PITUITARY IN THE RABBIT.

BY LYDA MAY DEGENER AND A. E. LIVINGSTON.

BOTH lobes of the thyroid, including the internal parathyroids, were removed from one group of rabbits which were allowed to live together with a corresponding number of controls for varying periods up to about six months after the operation and then killed. The pituitaries were carefully dissected out and weighed, and it was found that, for the controls, the average weight of the pituitary in milligrams per kilo of body weight of the rabbit was 11.4, and for the operated animals 17.6.

In another group the ovaries or testes were removed. The animals and controls were kept in the open air in the same pens as the thyroidectomized group and their controls and under precisely similar conditions with regard to feeding and attendance. Here the average weight of the pituitary, in milligrams per kilo of body weight, was, for the controls, 12.11 and for the operated animals 12.21. It would appear, therefore, that thyroidectomy, in the rabbit, is followed by hypertrophy of the pituitary, while castration is not.

STUDIES IN THE GENERAL PHYSIOLOGY OF SMOOTH MUSCLE

BY E. B. MEIGS.

THE fact that a tissue may swell in distilled water and lose weight in strong solutions of certain salts is insufficient to show that its cells are surrounded by semi-permeable surfaces. If the cell surfaces are

semi-permeable, this fact should show itself in, at any rate, the three following ways: (1) the tissue should respond accurately to the osmotic pressure of a series of solutions made up with different salts; (2) the tissue should respond accurately to the osmotic pressure of solutions made up with certain non-electrolytes; (3) the curves which represent the manner in which the tissue gains weight in hypotonic solutions and loses weight in hypertonic solutions should be at least roughly similar to such curves as can be obtained with artificial membrane osmometers. In a recent article the author has shown that the responses of frog's striated muscle to all three of these tests indicate that its fibres are surrounded by semipermeable surfaces, while the responses of the smooth muscle of the same animal indicate that the surfaces of its fibres are highly permeable to both sugars and salts.

The two classes of muscle found respectively in the adductor muscles and in the foot of lamellibranch molluscs swell in both isotonic and hypertonic salt and sugar solutions, and the curves which represent the gain in weight by the two tissues in hypotonic solutions are entirely different from those which can be obtained with membrane osmometers. It seems probable, therefore, that in muscle semipermeable surfaces surrounding the fibres are the exception rather than the rule.

THE INFLUENCE OF PREGNANCY ON THE CYCLIC CHANGES IN THE UTERUS.

BY LEO LOEB.

IN previous investigations I have shown that cuts made into the uterine wall or foreign bodies introduced into the lumen of the uterus of the guinea pig about four to eight days after ovulation will lead to the formation of placentomata. I have furthermore shown that extirpation of the corpora lutea at an early stage after ovulation will prevent the formation of the placentomata. In another series of experiments I showed that extirpation of the corpora lutea within the first week after ovulation leads to a marked decrease in the length of the period of the sexual cycle in the guinea pig. The corpora lutea inhibit, therefore, the rupture of the graafian follicles. In our new investigations we examined the changes which take place in the

uterine wall of the guinea pig during the different stages of the sexual period. There is a periodic change in the activity and in the morphology of these structures which corresponds to the various phases of the sexual cycle. With each new ovulation a new cycle of these changes begins. Now I found that if through early extirpation of the corpora lutea a new ovulation is accelerated simultaneously with the new ovulation a new cycle of changes sets in in the uterine wall. If, however, we permit pregnancy to develop in one horn we find, upon examination of the ovaries and of the uterine mucosa sixteen to twenty days after the previous ovulation, and after an early excision of the corpora lutea, the premature ovulation in the ovaries to have taken place in a similar manner as without simultaneous pregnancy, while in the uterine wall the setting in of the new cycle has been prevented through the presence of an embryo or of a placenta in the horn of the other side. We see, therefore, that although pregnancy does not prevent the early ovulation after previous excision of the corpora lutea it prevents the setting in of a new cycle in the uterine mucosa. Pregnancy exerts, therefore, an inhibiting influence on the cyclic changes of the uterine wall, while it does not affect the early ovulation taking place after excision of the corpora lutea.

THE PERIPHERAL ACTION OF CERTAIN DRUGS WITH SPECIAL REFERENCE TO THE LUNGS.

BY D. E. JACKSON.

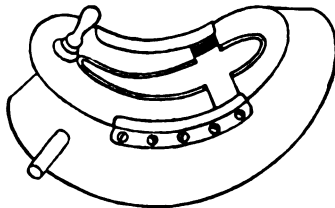
By the use of a specially designed metal shield which is placed between the pericardium and the right lung it is possible to obtain records of the lung volume changes in a spinal animal under the most favorable conditions. The chest of the animal may be bound immovably to the operating board if desired.

A method for maintaining the general blood pressure of an animal at a practically constant level was described.

In an animal prepared in this manner the injection of pilocarpine causes a constriction of the bronchi. Injection of epinephrine then causes a prompt dilatation, although the general blood pressure may undergo no change. This shows that bronchial dilatation by epine-

phrine, which under all ordinary conditions is accompanied by a marked rise in pressure, is in no wise dependent upon such rise.

Injection of considerable quantities of atropine leaves the bronchodilator nerve endings still sensitive to epinephrine long after the constrictor endings have been paralyzed. Sodium iodide and sodium camphorate have no effect on these endings. Agarcin has some depressing action on the constrictor endings. A strong muscular tonus produced by a number of substances may or may not be overcome by epinephrine, the question being apparently one of degree only.



Pilocarpine in small doses in a fresh spinal animal produces a slight broncho-constriction followed by a dilatation. If the adrenal glands be tied off, dilatation does not occur. This is analogous to paradoxical effects occurring elsewhere in the body under small doses of pilocarpine when the adrenal glands may be stimulated. Tyramine also appears to cause a bronchial dilation in a fresh animal if some initial constriction be present. This apparently is an epinephrine effect. Among ganglionic poisons nicotine appears to also cause dilation in fresh animals from increased adrenal secretion. But nicotine also has other and more obscure effects, a slow dilation having been observed (after pilocarpine contraction) in spinal animals after previous complete ganglionic paralysis.

Cholin chloride is fully as active as epinephrine in causing bronchial dilation. A similar but possibly slightly less extensive action is possessed by trimethylamine hydrochloride and by the 3 : 4-dihydroxyphenylethylmethylamine.

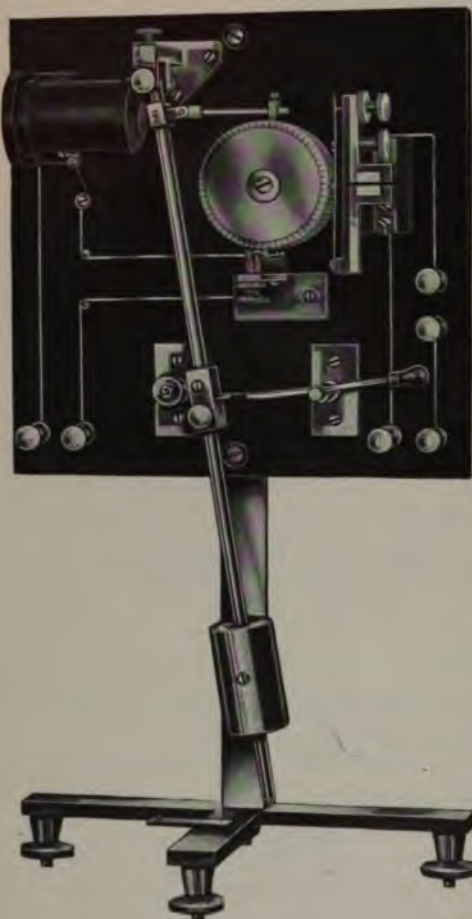
AN ELECTRICAL CLOCK.¹

BY W. T. PORTER.

A PENDULUM moves a pawl which each second turns a wheel one sixtieth of its circumference. Each movement of the wheel makes a

¹ I am indebted to Mr. C. E. Roy for his painstaking and intelligent assistance in carrying out this design.

contact at the platinum points seen beneath the wheel, and thereby sends a momentary current through a magnet which pulls upon the



pendulum near the point of suspension. This magnetic pull keeps the pendulum swinging at its normal rate.

On the rim of the wheel are five parallel tracks in which are set steel timing plates, 60, 30, 12, 2, and 1, respectively. As these plates are carried against the contact spring at the right of the wheel, a signal magnet records intervals of one, two, five, thirty, and sixty seconds.

The driving circuit is independent of the time circuit. Fine adjustments are everywhere provided. The signal contact spring may easily be moved along a traversing bar from one track to another. The instrument is readily portable. When the screw feet are adjusted to bring the end of the pendulum exactly above the point of the levelling device,

the pendulum will swing properly in a plane vertical to that of the three screw feet.

THE RELATION OF PULSE PRESSURE TO RENAL SECRETION.

BY ROBERT A. GESELL.

THE effects of changes of pulse pressure on the secretion of urine were studied on the intact kidneys of the dog. For changing the pulse

pressure the principle of air compression was used. An air chamber under mean blood pressure was connected indirectly with one or both renal arteries. The usual effect on pulse pressure of connecting the air chamber was to diminish the magnitude and decrease the suddenness of pressure changes.

Since the volume flow of blood and mean blood pressure are important factors in renal secretion, the relation of altered pulse pressure to these factors was determined. It was found that diminution of pulse pressure by this method had practically no effect upon mean blood pressure or volume flow of blood through the kidneys. Therefore any changes of secretion accompanying a change of pulse pressure must be ascribed to the effects of pulse pressure itself.

1. It was found that normal pulse pressure had a beneficial effect upon the secretion of urine.

2. That diminishing pulse pressure had a deleterious effect on secretion, sometimes lasting for an hour after subjecting the kidneys to normal pulse pressure.

3. In some instances, on connecting the air chamber with the renal arteries, the magnitude of the pulse pressure was unchanged, slightly diminished, or even increased as much as 15 mm. Hg. Yet in every case a copious flow of urine was abruptly stopped and held in check until the kidneys were again subjected to normal pulse pressure, indicating that in addition to magnitude of pulse pressure the suddenness of pressure changes may be of great importance in renal secretion.

4. The amounts of chlorides, urea, and total nitrogen eliminated during periods of normal pulse pressure were greater than during periods in which the pulse pressure was decreased.

5. In two experiments in which albumin appeared in the urine the amount eliminated during normal pulse pressure was less than during periods in which the pulse pressure was decreased.

THE FUNCTIONAL RELATION OF THE NERVE CELLS IN THE VASOMOTOR CENTRE.

BY W. T. PORTER.

A FORMER communication¹ introduced a new problem, namely, whether different vasomotor fibres influence all the bulbar vasomotor

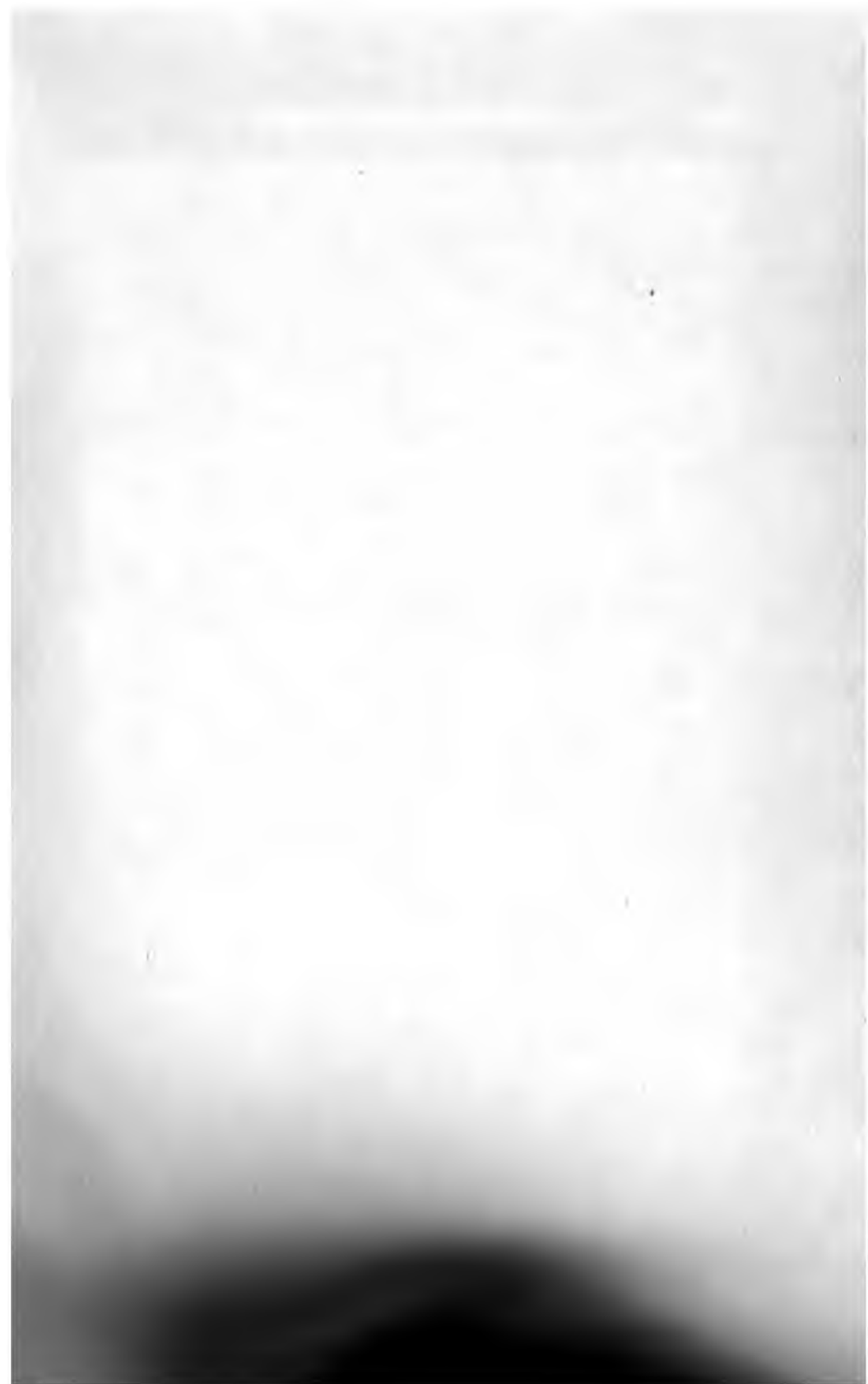
¹ PORTER and BEYER: this Journal, 1900, iv, p. 283.

cells alike, and reached the conclusion that the depressor nerve makes no special connection with the cells which control the vasomotor fibres of the splanchnic nerves. In the present investigation, which has had the assistance of Dr. A. L. Meyer, I have endeavored to determine whether the depressor, the sciatic nerve, and the nerves of the brachial plexus are connected with the same nerve cells in the bulbar vasomotor centre.

When the centre is made anæmic in the rabbit by clamping the carotid and the vertebral arteries and raising the animal's head higher than the feet, the reflex fall or respectively the reflex rise on stimulation of the central end of the depressor, sciatic, and brachial nerves disappears. On placing the rabbit in the horizontal position and removing the clamp from one carotid artery, the reflex returns. As the vasomotor cells gradually recover, the reflexes gradually increase to their normal value. A "recovery curve" is thus obtained in which the changes in blood pressure are plotted as ordinates, while the time from the first feeble reflex to full recovery is used as the abscissa.

If the recovery curves on stimulation of the depressor brachial and sciatic nerves pursue each a different course, the nerves cannot be connected with the same bulbar cells.

In the experiments here reported the recovery curves were identical. These several nerves therefore connect with the same vasomotor cells, unless we make the not very probable assumption that all the vasomotor cells are affected alike by the experimental anæmia.



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